MikroWin Version 4.18
Mithras Software Version 0.05
Mithras Driver Version 0.16
July 2002

LB 940
Multilabel
Reader
Mithras

Id No.: 38099BA2
Rev. No.: 00

Contents

Explanation of LED's and Beeps

LED	***Instrument status***
lights up **green**	Instrument OK and connection to PC OK
lights up **yellow**	Instrument OK, no connection to PC
flashes **yellow** + 1 short beep	New CAN is installed after power on of instrument
lights up **yellow** + 1 longer beep	CAN correctly installed
lights up **red**	Shortly after power on of the instrument (during initialization)
flashes **red** + 2 short beeps	Error after power on of instrument / CAN module not correctly installed

Note

The MikroWin *Lite* software is supplied as standard version with the Mithras instrument. This software version does not include all functions described in this user guide and in the MikroWin user guide. The versions *Advanced I* and *Advanced II*, which include additional functions, are also available.

Typographical Conventions

<**Add formula** >, <**OK**>, <**Close**>	**Buttons** are printed inside angular brackets in boldface type
Menu **File**, **Open** dialog box	**Menu titles** and **dialog boxes** are printed in boldface type
File | Open, **Options | Read**	**Menu items** are also printed in boldface type; menu and submenu item are separated by a vertical line.

1. Safety Instructions

This User Guide includes information and warnings that have to be observed by the user to ensure safe instrument operation.

Please do always act according to the following safety instructions, before as well as during operation of the system! Before taking the instrument into operation, it is absolutely essential to read the user manual, as otherwise the safety of instrument and operator cannot be ensured.

The **Multilabel Reader LB 940 *Mithras*** has been manufactured in accordance with the safety requirements for electronic and medical measuring systems. If the law lays down regulations on the installation and/or operation of sample measuring system, then it is the operator's responsibility to adhere to them.

The manufacturer has done everything possible to guarantee that the equipment functions safely, both electrically and mechanically. The user has to make sure that the instrument will be set up and installed properly to guarantee safe operation.

The instruments have been tested by the manufacturer and are supplied in a condition that allows safe and reliable operation.

- ❒ The instruments may only be operated by personnel who have been trained on the use of the system. It is strongly recommended that all users read this manual prior to use.
- ❒ **Remove the transport safety devices before you turn on the instrument.**
- ❒ Use the instrument only for the designated application.
- ❒ **BERTHOLD TECHNOLOGIES** assumes no liability for any damages, including those to third parties, caused by improper use or handling of the instrument.
- ❒ The user is responsible for connecting the instrument in accordance with the valid regulations for electrical instruments.
- ❒ The instrument is provided with a 3-pole grounded plug. If your wall outlet does not allow connection of a 3-pole plug, have a suitable wall outlet installed by qualified personnel or use an adapter for safe grounding. Please observe the safety specifications of the grounded plug.

- ❒ The instruments are designed according to the IEC 1010-1 or EN 610 10-1 regulations for electrical measuring systems.
- ❒ Do not open any instrument doors as long as the instrument is in operation.
- ❒ Service and repair work may be carried out by qualified personnel only.
- ❒ The operator may only perform the maintenance work described in this user guide.
- ❒ Use only parts described in this manual for servicing.
- ❒ Disconnect power supply before opening the instrument.
- ❒ Pull the power cord to disconnect instrument from power supply.
- ❒ Turn instrument off before pulling the power cord.
- ❒ If the hood is removed, the safety provisions are no longer complied with. Watch out for moving parts. Inside the instrument temperatures may be present which may cause burns. Some parts of the instrument may still be hot after power off, although this is not immediately obvious.
- ❒ The electronic unit of the detector generates high voltage. Do not touch it during operation!
- ❒ **Caution: Risk of explosion if battery is inserted incorrectly. The battery may be replaced only using the same battery type or a type recommended by BERTHOLD TECHNOLOGIES by a person authorized by BERTHOLD TECHNOLOGIES. Spent batteries have to be disposed off in accordance with the manufacturer's instructions.**
- ❒ The instrument has to be set up such that the mains switch is easily accessible.
- ❒ If you can see that the instrument has become unsafe to use, switch it off and disconnect it from power supply.
- ❒ If liquid gets inside the instruments, pull the power cord. Clean the unit or have it cleaned by an authorized service center.
- ❒ Protect yourself from electrostatic charge, as discharge could damage sensitive instrument parts, especially sensitive parts of the computer and electronics cards.
- ❒ The system always has to be primed with solutions recommended by the kit manufacturer.
- ❒ Use only reagents recommended by the kit manufacturer.
- ❒ Use reagents only in accordance with the kit manufacturer's instructions.

- ❒ Do not use any flammable or explosive solutions or liquids whose mixture is flammable or explosive.
- ❒ Waste (when priming/washing the tubings) always has to be disposed off properly: if a waste pump is installed, a bottle has to be connected. If no waste pump is present, a suitable prime plate has to be placed below the injectors during priming/washing.
- ❒ Injector solutions may be pumped back only if the appropriate reagent bottle is connected.
- ❒ Observe all statutory requirements for handling biological waste, reagents and patient samples.
- ❒ **The operator is responsible for the use of reagents.**
- ❒ The ***Mithras*** instrument should be shipped in its own case. During transport the plate slide has to be secured by a safety screw.
- ❒ For instrument cleaning, please refer to the respective sections in this manual.
- ❒ Reliable instrument function can be guaranteed only when original spare parts are used.

The tests and service work recommended by the manufacturer has to be performed to make sure that the operator remains safe and that the instrument continues to work correctly. Any service and maintenance work not described in this user guide has to be performed by authorized service personnel.

Special Spare Parts

These spare parts are essential for safe operation of the instrument: always use original spare parts supplied by the manufacturer or an authorized distributor.

Fuse	3A/T 5x20 type 179500 (ELU)	ID No. 12259 or
	3A/T 5x20 type 19198 (Wickmann)	ID No. 12259
Power supply	100-240VAC; 24V/6.5A type JWS-150-24 (Lambda)	ID No. 34846
Lithium battery	3V/220mAh type CR2032 (Varta)	ID No. 17391

2. Quick Reference Guide

This Quick Reference Guide will lead you step by step through the various work processes, i.e. getting started, running measurements and performing evaluations. It provides a quick overview on how to work with the software and the instrument.
Mithras is a very versatile instrument for different types of applications in the field of luminescence and fluorescence measurement. To meet the needs of different users, this Quick Reference Guide is structured as follows:

- The Quick Reference Guide is divided into separate sections, so you can choose those chapters that are important to you: getting started, software installation, new definition of counting parameters, creating counting parameters on the basis of existing parameter files, measurement and evaluation.
- In each section you are guided through the various procedures step by step. These steps are numbered consecutively in each section. Explanations on the individual steps are added in small type font.
- Explanations on the various types of measurement are highlighted specifically.
- For your convenience, illustrations are placed directly next to the respective text.

2.1 Getting Started

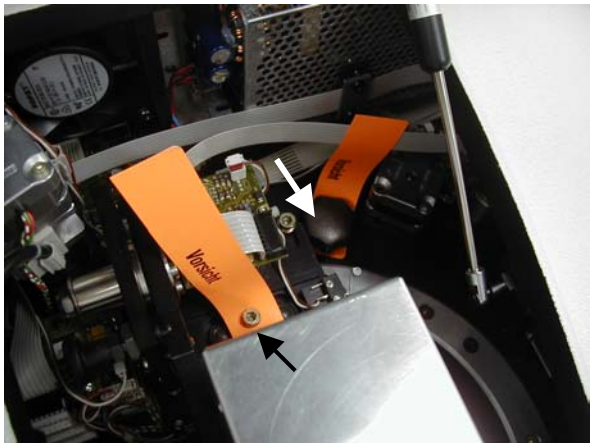
(see chapters 4.1 – 4.4)

1. Unpack and set up ***Mithras***.

2. Unscrew instrument cover and open it.

3. Unscrew transport safety screw fixing the photomultiplier and the plate slide and keep transport safety screw in a safe place (see chapter 4.4). Do not take instrument into operation before you have removed the transport safety devices, as otherwise severe damage may occur.

4. Close instrument cover again and fix it with screws.



Mithras rear panel

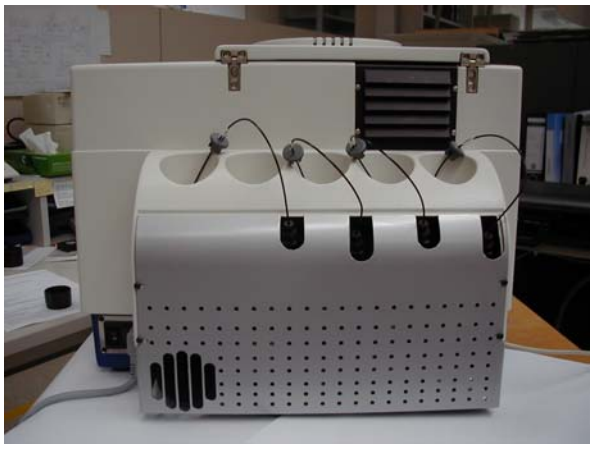
5. Check if the power supply is within the permissible range of the operating voltage of the ***Mithras***. Connect instrument **only if this is the case!**

6. Install hardlock supplied on parallel port of PC.

7. Connect ***Mithras*** to serial port of PC.

8. Connect instrument to power using the power cord supplied.

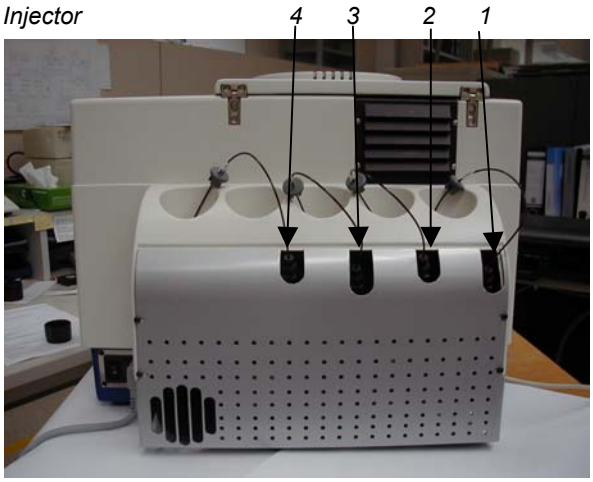
9. If a *waste pump* is installed in the instrument, connect tubing to the reagent outlet and pass it into a suitable bottle to collect the wash solution.



10. Connect reagent tubing to injectors using a screw cap.

The injectors are located on the right side of the instrument and are numbered as follows (from left to right): 1 = injection into the well located 1 position before luminescence reading position, 2 + 3 = injection in luminescence reading position, 4 = injection in top reading position.

Rear panel

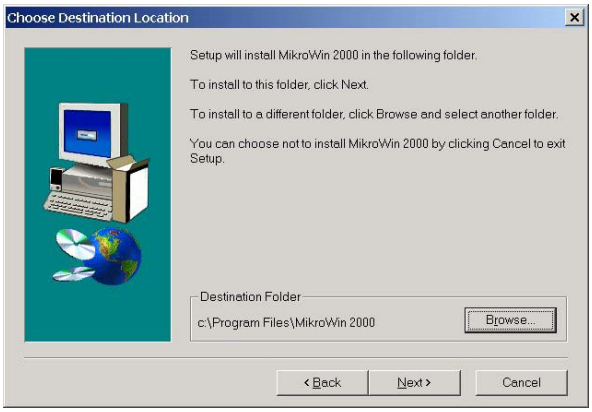


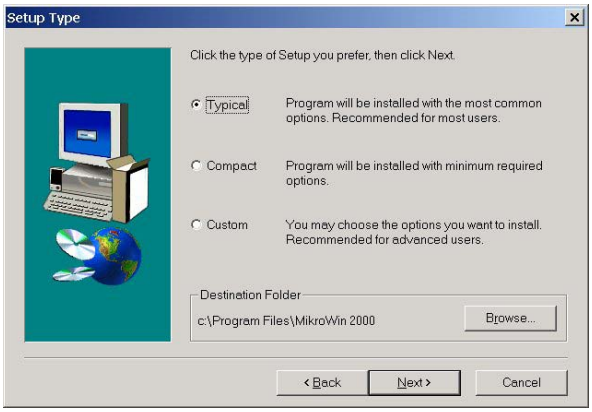
11. Turn instrument on.

2.2 Installation of MikroWin and Driver Software

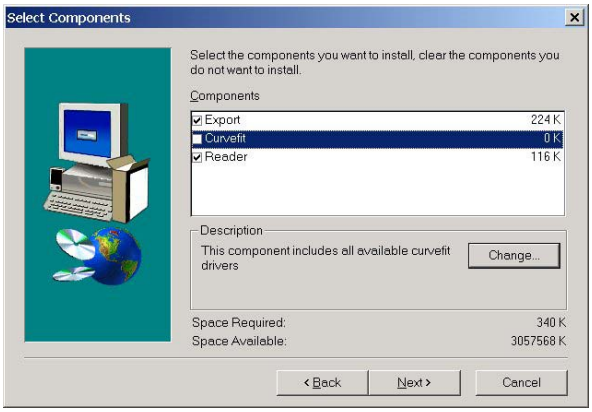
2.2.1 MikroWin2000 Installation

1. Close all *Windows* applications before you start installing the software.
2. Insert **MikroWin2000** CD into CD tray. The installation routine starts automatically.
3. Select language and confirm with <**OK**>. The setup assistant is started.
4. Enter name and company and click <**Next**>.
5. Choose destination location (see screen shot to the right).
   The following path is defaulted
   C:\Program Files\MikroWin2000.
   If you wish to install the program to another folder, click <**Browse**> and select another folder.
6. Click <**Next**>.
7. Select the **setup type**.
   We recommend that you choose **Typical** for your first installation to ensure that all program components are installed. If you are familiar with the system, you may choose **Custom** to select the components you need for your application.
8. Click <**Next**>.





9. Select the desired components or deselect those components you don't want to install.
   The option **Curvefit** is not really needed for working with ***Mithras*** and may be deselected.
10. Click <**Next**>.



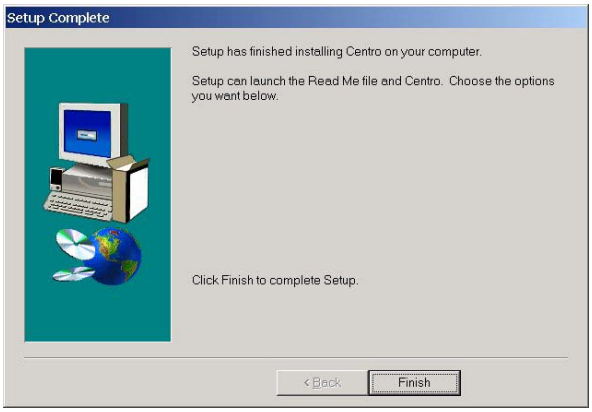
11. Add program icon to the ***MikroWin2000*** program folder.

12. Click <**Next**>. Installation is carried out and successful completion is indicated.

13. Click <**Finish**>.

2.2.2 Driver Software Installation

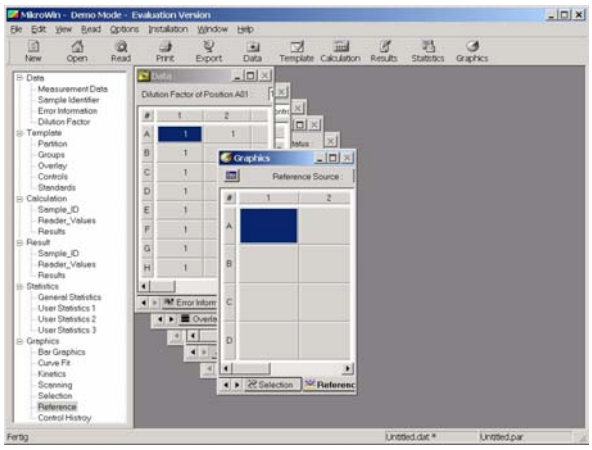
1. Insert CD containing the ***Berthold Technologies*** **driver software**. The installation routine starts automatically.
2. Select language and confirm with <**OK**>. The setup assistant is started.
3. Click <**Next**> in the **Welcome** dialog box. The driver is automatically installed to the ***MikroWin2000*** folder and the appropriate subdirectory.
4. Upon successful driver installation, the **Setup Complete** dialog box appears. To be able to work with the new software you have to restart your computer.
5. Choose "**Yes, restart computer**" and click on <**Finish**>. Your computer is restarted and all program components are installed in the *Windows* operating system.



6. Click on the program icon  to start ***MikroWin2000***.
7. The main window of ***MikroWin2000*** is displayed.

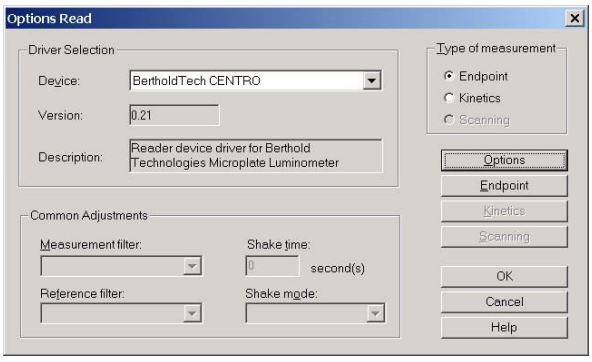
   The main window with menu bar, tool bar and navigation bar is displayed. It includes all menus and menu items. In addition, all child windows (data, template, calculation, result, statistics, graphics) are displayed; these windows can be minimized or maximized as needed.

   The navigation bar can be deselected in the View menu. See ***MikroWin2000*** software User Guide.
8. Set up driver. Select menu item **Installation | Driver** and verify or define parameters (see chapter 5.2.3).



9. Select menu item **Options | Read**. The **Options Read** dialog box is displayed.
10. Open the **Device** drop-down list and select the item **BertholdTech Mithras**.

    The respective drivers and pre-defined parameter files are loaded. The other text boxes in this dialog box are filled in automatically.
11. Define excitation and emission filter on the **Instrument** menu and install filters into the instrument accordingly (see chapters 5.4.8 and 5.4.9).



2.3 Definition of Measurement Sequence and Parameters

There are two alternative ways of defining the measurement sequence and parameters:

a) Measurement and evaluation parameters are defined completely new (see section 2.3.1).

b) An existing parameter file is used and the parameters are modified as needed. Either you use the parameter files supplied by ***Berthold Technologies***: each includes a typical run for each type of measurement (see section 2.3.2). Or you use parameter files you have created yourself and edit this file accordingly.

Please note: A saved parameter file includes: selected wells, individual operations and evaluation parameters (e.g. calculation formulas).

2.3.1 Creation of New Measurement Sequence and Definition of Parameters

1. In the ***MikroWin2000*** main window, select the menu item **Edit | Reset**. **Untitled.par** is displayed as temporary file name in the status bar.

2. Select **Options | Read**. The **Options Read** dialog box is displayed.

3. Select **BertholdTech Mithras** from the **Device** drop-down list.

4. Click on <**Options**>. The **Options** dialog box appears showing two tabs: **Samples** and **Measurement**.

Well selection (see chapter 5.5.3)

5. On the Samples tab, select plate type, desired wells and measurement order (by rows or by columns).

Select plate type (6, 12, 24, 48, 96, 384 or 1536 wells).

In the row Plate Mode, enable the item Measurement and then select the wells for the measurement.

Select desired injector in the row Plate Mode and then select the wells into which the respective injector is to dispense.

Presentation

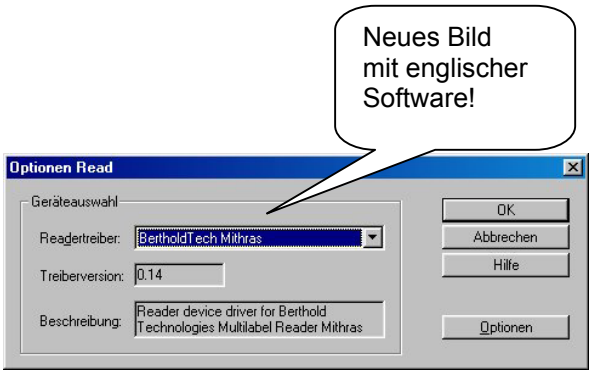
Wells selected for reading: 

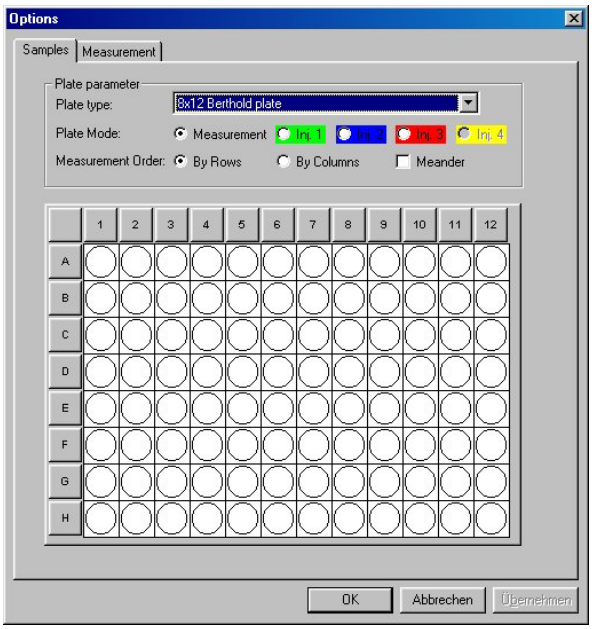
Wells selected for injector 1: 

Wells selected for injector 2: 

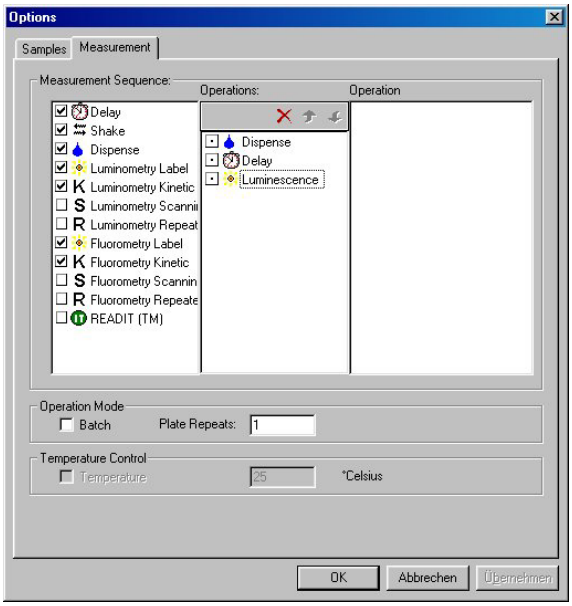
Wells selected for injector 3: 

Wells selected for injector 4: 

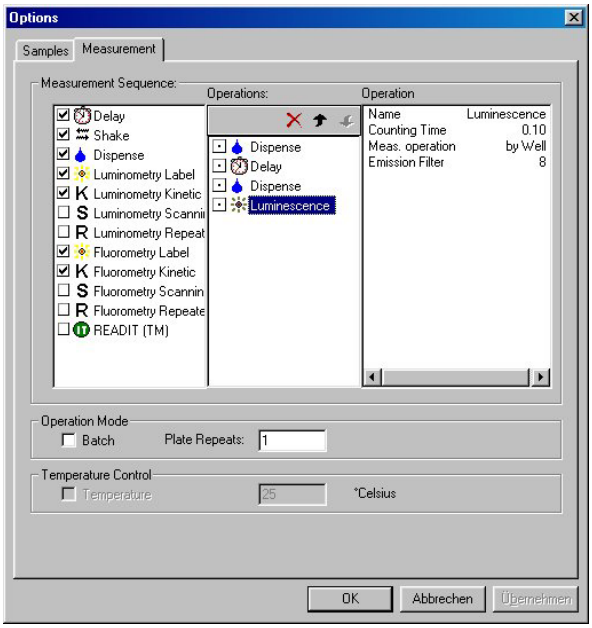




Define measurement sequence (see chapter 5.5.4)



6. After you have selected the wells, click on the **Measurement** tab to define the measurement sequence with the individual operations.
7. Copy the respective operations one after the other into the **Operations** column:
   - double-click the desired operation in the left column.
   - enter the desired parameters in the respective parameter box and click <**OK**> to confirm you entries.
8. In the **Operations** column you may change the sequence of selected operations by clicking on the  and  buttons.



9. Select **Operation Mode** (if needed):

   Batch: In this mode several plates can be read in succession using this protocol.

   Plate Repeats: The same plate can be read several times in succession. Enter the number of repeats in this box.
10. Define temperature, if your instrument includes a temperature control (input option: 15° - 45°C). ***Please note:*** You can enter a target temperature in the instrument which is *at least* +5°C above room temperature, i.e. if the room temperature is 20°C the minimum instrument temperature is 25°C.
11. Confirm entries with <**OK**>.

Define evaluation parameters (see chapter 5.5.6)

Evaluation parameters are selected using the options on the **navigation bar** or via the **tool buttons** and defined on the respective matrices. Generally, these pre-defined parameters hardly have to be modified for simple luminescence and fluorescence measurements. Check the following matrices anyway and make the required changes.

You can add matrices to each child window or deselect these matrices (**Options | Matrix...**) and change the parameters for measurement and evaluation on each matrix (by well, by row or column or for the entire microplate). The exact procedure is described in the ***MikroWin2000*** User Guide.

12. Select **Data** and enter results, sample identifier, error information and dilution factors on the matrices.

13. Select **Template**.
    to change the partition of the sample matrix and define controls and standards. The other matrices are partitioned accordingly.

14. Select **Calculation**.
    to define calculation formulas for the individual matrices.

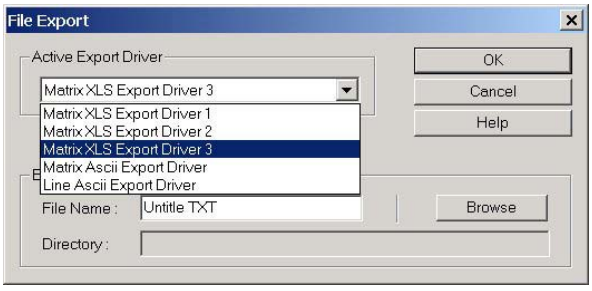
15. Select Results.
    Results and calculated values are displayed after measurement.

Define export parameters (see chapter 5.3)

16. Prerequisite: Install and set up export driver (see also chapter 5.2.3).

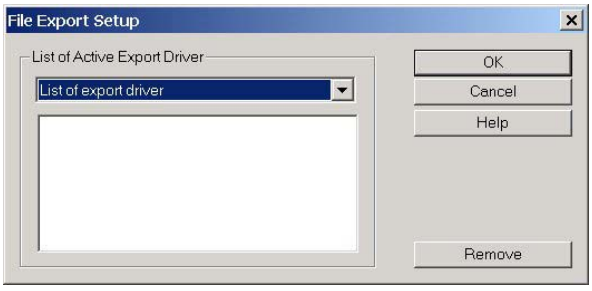
17. Select **Export**. The following functions are file-specific, i.e. they are valid only for the **active** parameter file.

18. In the **File Export** dialog box select the item you want, e.g. **Matrix XLS Export Driver** if you want to create an Excel file.



19. Confirm your selection with <**OK**>.

20. To export the data automatically after measurement, select **File | Export Setup...** in the main window. The **File Export Setup** dialog box is displayed.



21. Select desired export driver. It has to be the same as the one selected in the **File Export** dialog box.

Save parameter file (see chapter 5.5.2)

As soon as the measurement sequence has been generated and all parameters mentioned above have been defined as needed, save the data to a parameter file.

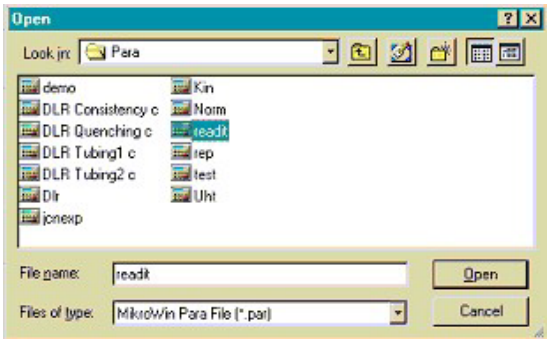
22. Select **File | Save As ...** .
23. Enter file name (extension .PAR) and confirm with <**OK**>.

2.3.2 Editing Parameter Files

In this case, an existing parameter file is modified. Basically, all **MikroWin2000** parameter files can be edited.

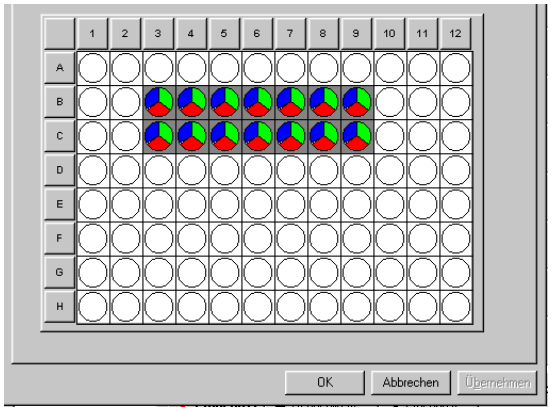
The basic parameter files supplied by ***Berthold Technologies*** include typical measurement sequences of some major measurement types. Using this data, you just have to make a few changes to define the measurement sequence for your own requirements. A parameter file includes the selected wells, the pre-defined operations and the evaluation parameters (e.g. calculations, result presentation and export). The type of measurement is indicated in the file name. Depending on your instrument setup, it may happen that not all defaulted parameter files can be utilized to the full extent (see chapter 5.1).

Save the changes to these files under a *new name* in order to keep the basic parameter files!



1. Select **File | Open** in the main window.
2. Select the file type ***.par** in the **Open** dialog box.
3. Select the desired parameter file and confirm with <**OK**>.

   A parameter file is active until a new one is opened or until you exit the program. The name of the open file appears in the bottom right corner of the screen.



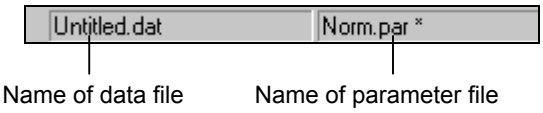
4. Select **Options | Read ...** and in the **Options Read** dialog box click on the <**Options**> button. The **Options** dialog box appears. The wells selected for measurement and injections are displayed on the **Samples** tab.
5. Change well selection as needed (see page 11; for more details see chapter 5.5.3).
6. Select **Measurement** tab. The pre-defined measurement sequence is displayed.
7. Change measurement sequence as needed (see page 12; for more details see 5.5.4).

   For differences in the individual types of measurement see chapter 5.5.9.

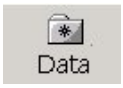
Verify evaluation parameters

Evaluation parameters are selected using the options on the **navigation bar** or via **tool buttons** and set on the matrices (see page 13). The name of the open parameter file appears in the bottom right corner of the screen.

For more information please refer to the ***MikroWin2000*** user guide.

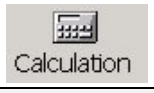


8. Select **Data** and edit matrices, if necessary.



9. Select **Template** and edit matrices, if necessary.

10. Select **Calculation** and edit matrices, if necessary.



For differences between the individual types of measurements see chapter 5.5.9.

11. Select **Results**.

Matrices are displayed depending on the pre-selected calculation matrices. Calculated results are displayed depending on the pre-defined formulas.

12. Define export parameters (see page 13).

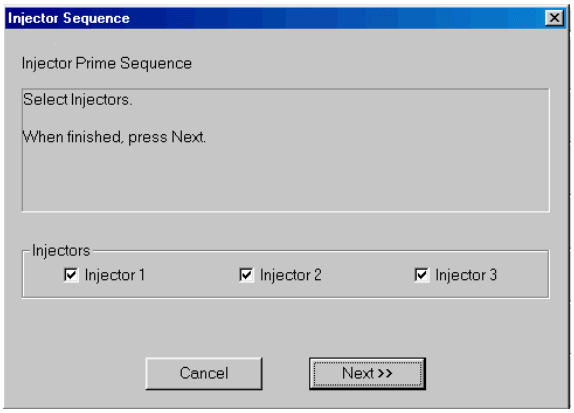
13. Save parameter file (see page 14)

As soon as the measurement sequence has been created and all parameters have been defined as needed, save the parameter file under a *new name* to keep the basic parameters.

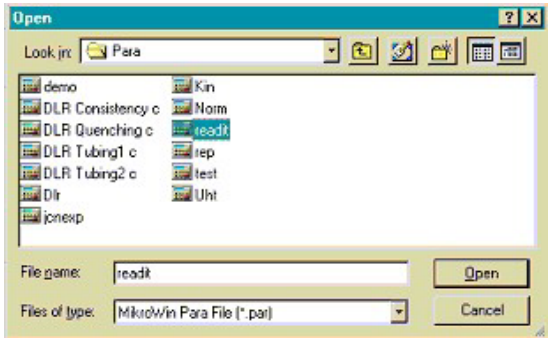
- Select **File | Save As ...**.
- Enter file name (extension .par) and confirm with <**OK**>.

2.4 Measurement and Evaluation

1. The ***Mithras*** instrument has to be connected completely and powered on.
2. Prime injector tubings used (see chapter 5.4.3):
   - Select **Instrument | Prime**.
   - In the **Injector Sequence** dialog box, select injectors to be primed and click <Next>.
   - If no waste pump is connected, you are prompted to place a prime plate in the plate loading area. A prime plate always has to be inserted for injector 4 . Injector 4 always has to be processed separately from injectors 1 to 3. Then click <**Next**>.
   - Connect the reagents to the respective injectors. Click <**Next**>. Then 13 shots per injector are carried out.
   - The end of the prime process is displayed. Remove prime plate, if you have used one, and click <**Close**>. The **Injector Sequence** dialog box is closed.



3. Open parameter file (*.par) for measurement (see chapter 5.5.2). The matrices associated with this parameter file are displayed (depending on your choice of the menus **Data**, **Template**, **Calculation**, **Results**).



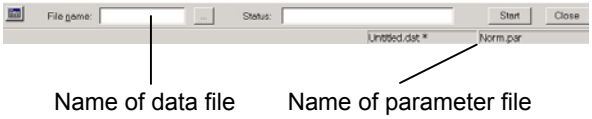
4. Click on the **Read** button. A bar with input fields and buttons appears below the displayed matrix.

   The *name* of the parameter file used is displayed in the bottom row.

   Click  to open the parameter file and you can edit the default settings, etc.

   File name: enter a file name for the data.

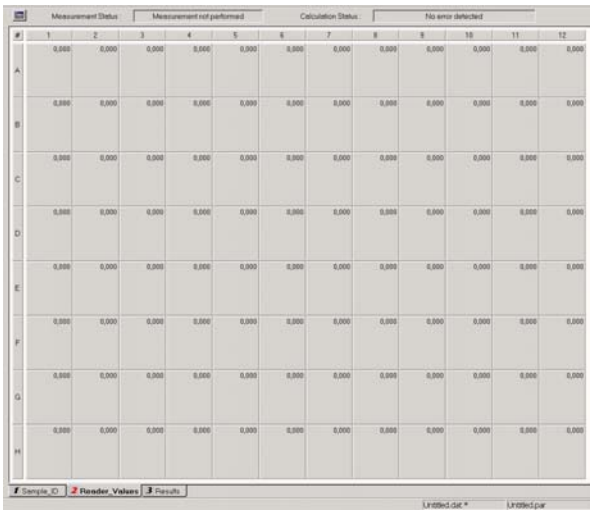
   Click <**Start**> to start the measurement after you have entered a file name for the data.



5. Enter file name for the data.

6. Click <**Start**>.

7. The plate tray opens. On the screen appears the prompt to insert the microplate to be read.
8. Insert plate (A1 at the rear left) and click <**OK**>.
9. The plate is moved into the instrument and the pre-defined measurement sequence is started.
10. During measurement, the data already available and the calculations can be viewed on the matrices of the menu **Results**.
11. Upon completion of the measurement the plate stays in the instrument to protect it from external influences. To unload it, chose the command **Unload Plate** on the **Instrument** menu.
12. If **Batch** has been pre-selected, the system expects the next microplate which is being read using the same parameters. Insert plate and click <**OK**>.
13. Results are presented in the **Graphics** window.



3. System Description

3.1 Overview

The ***Mithras LB 940*** is a Multilabel Reader for various fluorescence and luminescence measurements on microplates. Up to 4 injectors may be used. The strength of Mithras is its **versatility**, as well as the fact that this instrument has been **optimized for every measurement mode** and, therefore, supplies excellent results. Clear device design, simple handling as well as instrument control and data evaluation by the MikroWin2000 software make **Mithras** an indispensable measuring system in the (research) laboratory.

Fields of application

Fluorescence measurements	high sensitivity due to improved optics
Luminescence measurements	highest sensitivity due to optimized optics
FRET	measurement of the energy transfer between 2 fluorescent molecules
BRET	measurement of the energy transfers from a luminescence reaction to a fluorescent molecule

An extremely low-noise photomultiplier is used for these measurements. Highest sensitivities can be obtained due to using of single photon counting technology. The reading accuracy is further enhanced by using special methods to reduce crosstalk (light transfer between neighboring samples). The kinetics of fast luminescence and fluorescence reactions can be traced accurately by using ultra fast amplifiers.

Optimization for various measurement modes

- ❒ Depending on the selected measurement mode, the photomultiplier may move to three different positions to get to the optimum reading position
- ❒ The entire optics is height-adjustable to compensate for different heights of the microplates. In this manner, the microplate is automatically moved up to the photomultiplier, ensuring that there is no gap between photomultiplier and microplate.
- ❒ The instrument includes separate devices for excitation and emission filter with replaceable single filters.

Moreover, universal application of the ***Mithras*** is ensured by the following features:

- ❒ Free choice of plate formats (96 and 384 wells) with automatic setting of the optimum light path; 6, 12, 24, 48 and 1536-well plate formats are also freely selectable if the option automatic plate height adjustment is available.
- ❒ Use of up to 4 injectors with freely adjustable volume and adjustable injection force (JET injection technology)
- ❒ Shaking functions
- ❒ Temperature control (optional)
- ❒ Barcode reader (optional)
- ❒ Connection to lab automation systems (optional)
- ❒ Comprehensive software for instrument control and data evaluation (MikroWin2000)
- ❒ In addition to the software, basic parameter files are supplied for various measurement modes: kinetics measurement and repeated measurement for luminescence and fluorescence, standardized luminescence and fluorescence measurements, Dual Luciferase Reporter Gene Assay, Readit™ etc.

3.2 Reader Unit

The ***Mithras LB 940*** is a desktop instrument with small footprint. It can be set up on any lab workplace

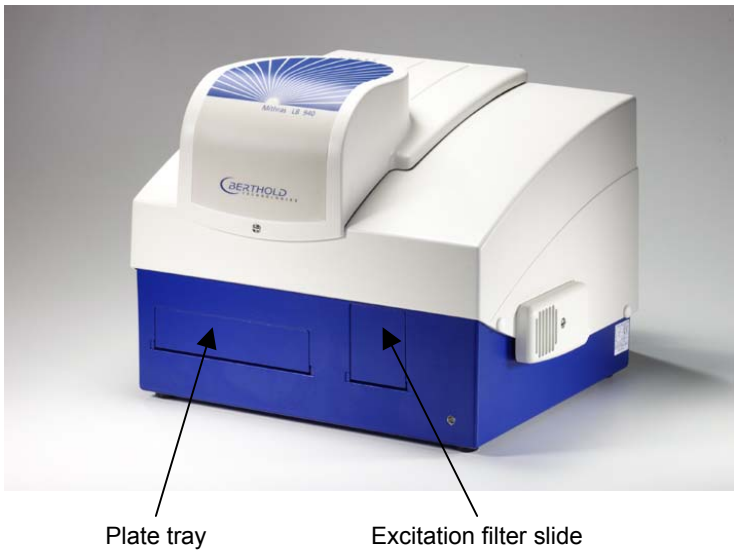


Figure 3-1: LB 940 Mithras

NOTE

To obtain good and consistent results, please keep the following in mind:

- ❒ Do not expose instrument to direct sunlight.
- ❒ Set up instrument in dry rooms.
- ❒ Open plate tray only for loading or cleaning to keep light and dust out.
- ❒ Open instrument cover only for cleaning. Prior to a measurement, the instrument cover has to be closed light-tight for a longer period of time.
- ❒ Keep plate tray free from dirt.
- ❒ Absorb reagents spilled inside instrument immediately using a clean and dry household tissue and then clean the respective location.

3.2.1 Plate Tray

The instrument front panel includes the plate tray. It can be opened and closed under control of the ***MikroWin2000*** software.

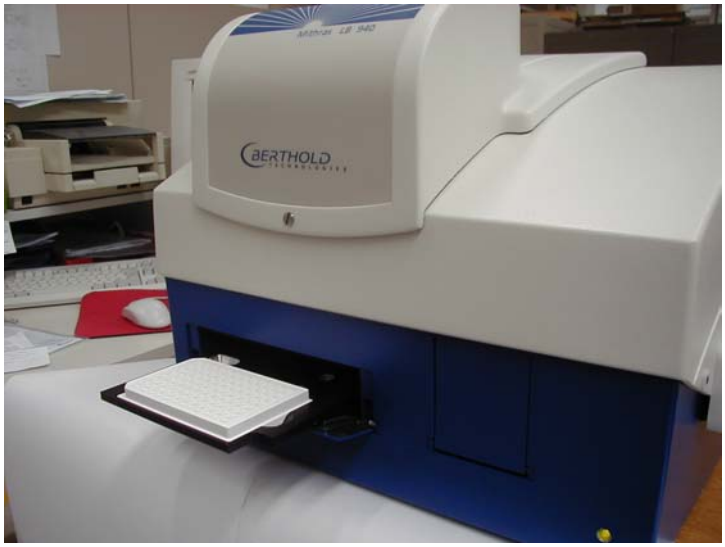


Figure 3-2: Open plate tray

Select **Instrument | Unload Plate** to open the plate tray; then place the microplate on the tray (during priming or washing a prime plate, if no aspiration pump is installed). Position **A1** of the microplate has to be in the rear left corner. Load the microplate such that it rests completely on the plate tray and is fixed by holders.

CAUTION

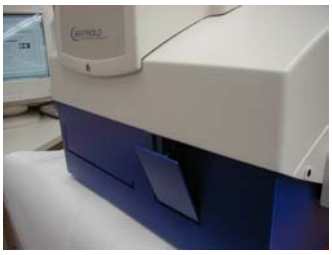
Incorrectly loaded microplates may cause damage or lead to false results.

Select **Instrument | Load Plate** to move the plate slide into the instrument and to close the plate tray light-tight.

3.2.2 Excitation Filter Slide

To the right of the plate tray there is a compartment containing the excitation filter slide (instrument front panel, Figure 3-1). To replace or clean the filter you have to eject the slide via software.

Proceed as follows



- ❒ Select **Instrument | Excitation Filter Slide**.
- ❒ In the **Excitation Filter Slide** dialog box, click on the button <**Eject Slide**>. The door opens slightly and the slide moves out a bit.
- ❒ Open the door all the way by hand and pull the filter holder out at both metal pins (bottom and top at filter holder).
- ❒ Clean or replace filter.
- ❒ If you replace the filter, drag the respective filter into the **Excitation Filter Slide** dialog box to the appropriate positions. See the explanations in chapter 5.4.8.
- ❒ Push in filter holder all the way into the slide.
- ❒ Click <**OK**> in the **Excitation Filter Slide** dialog box. The slide moves all the way into the instrument and the door is closed again.

3.2.3 Components Inside the Instrument

Open the screw on the instrument cover and raise the cover to have access to the inside of the instrument. The cover is held in place by a gas pressure spring.

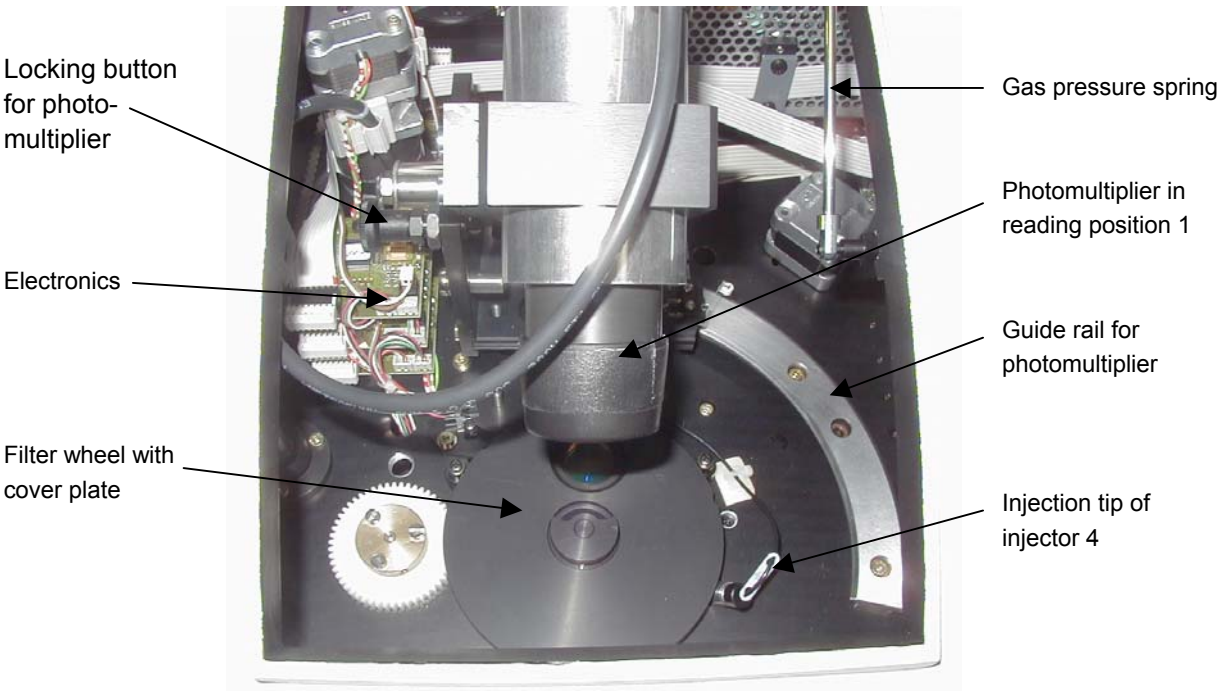


Figure 3-3: LB 940 Mithras with raised cover

Inside the instrument you find the photomultiplier, the emission filter wheel, the electronics with the high voltage generation, the injector tubings and tips, the toothed belt for height adjustment of the entire optics and the microplate slide which is moved to the reading and injection positions during measurement.

3.2.4 Photomultiplier

The photomultiplier measures light with high sensitivity and low noise. The spectral sensitivity is within a range that is suitable for all bio- and chemiluminescence applications.

The photomultiplier operates as an ultra-fast photon counter. The photo electrons released from the photo cathode by the light quanta are multiplied via the dynode chain; at the anode, these photons trigger a fast pulse with a rise time of a few nanoseconds. These counts are amplified by a very fast amplifier. Low-energy single counts created by the noise of the photomultiplier are suppressed by a threshold discriminator. The single counts are counted digitally; their total number is directly proportional to the emitted quantity of light.

So-called counts (cts) are used as unit of measure for the raw data.

The photomultiplier can be raised in order to remove the emission filter wheel. To do this, pull off the locking button on the right side of the photomultiplier (see Figure 3-3 and 3-4a and b).

Three reading positions of the PM

The photomultiplier with counting head is mounted on a guide rail (1/4 circular arc), so it can be moved to the optimum position depending on the measurement to be performed:

M1. For luminescence measurements and BRET
M2. For fluorescence measurements from below (clear plates!).
M3. For fluorescence from above

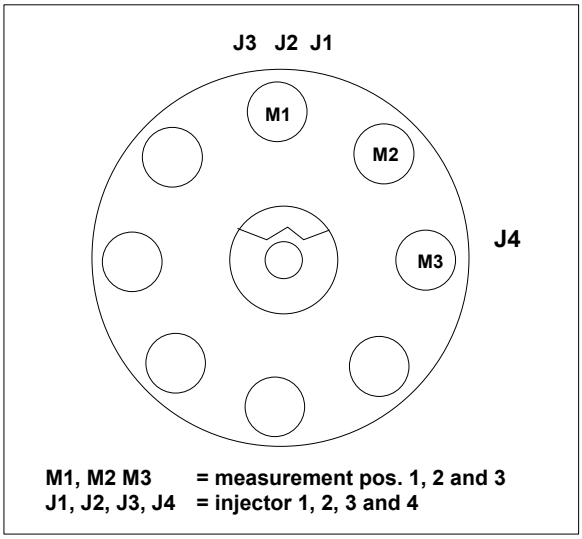


Figure 3-4a: Reading positions of PM and injector positions

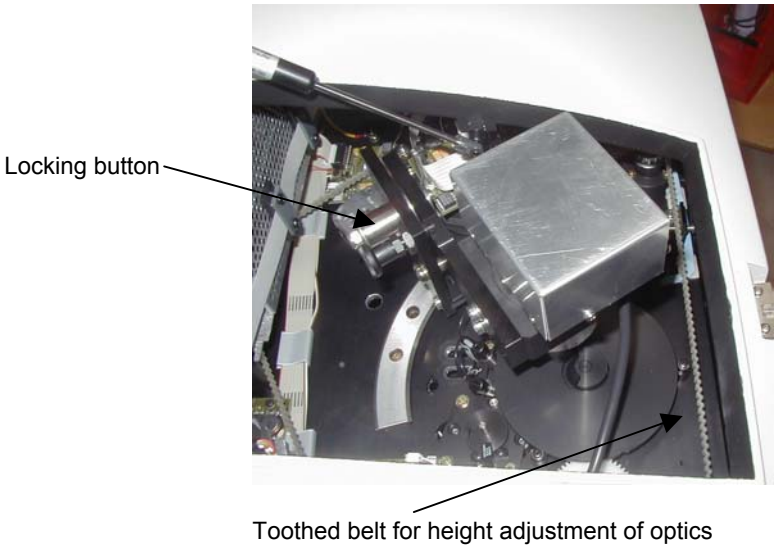


Figure 3-4b: Photomultiplier in bottom reading position

Four injector positions (see also chapter 3.2.6 Injectors)

J1	pre-position luminescence
J2 and J3	reading position luminescence
J4	reading position fluorescence

3.2.5 Emission Filter Wheel

The emission filter wheel located directly below the photomultiplier can accommodate up to eight different emission filters. The desired filter is moved to the respective reading position (1, 2 or 3) under control of the software. See also chapter 5.4.9.

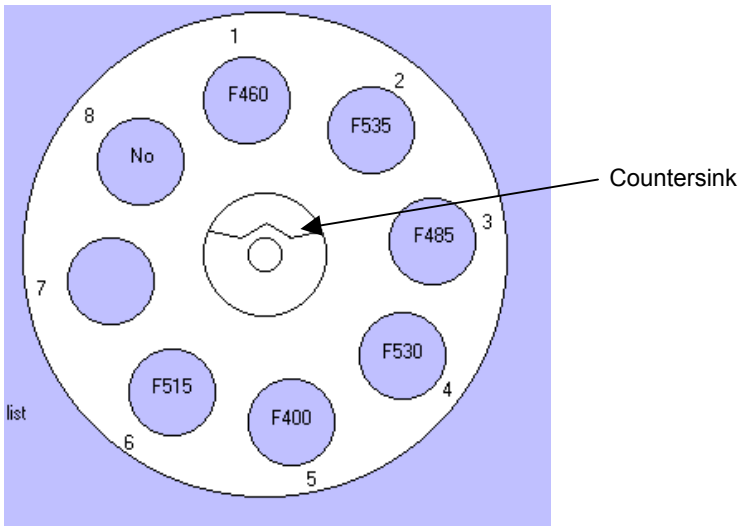


Figure 3-5: Filter positions on emission filter wheel

Proceed as follows to replace the filter wheel

❒ Open instrument (open screw and raise cover).

Photomultiplier raised, cover plate accessible

❒ With the instrument turned on, select the menu item **Instrument | Emission Filter Wheel**. The **Emission Filter Wheel** dialog box appears showing a schematic presentation of the filter wheel.

❒ Click on the <**Position Wheel**> button. Then the filter wheel is turned into the replacement position. This position is extremely important and should not be twisted when taking out the wheel. The photomultiplier is located in reading position 1, all the way at the back.

Cover plate removed, filter wheel accessible

❒ Pull off locking button (to the right of the photomultiplier) and raise the photomultiplier.

❒ Turn cover plate such that the 3 round countersinks are flush with the dowel pins, then take it off from above.

❒ Take out filter wheel and make sure that the base plate is not twisted.

Replacing filters

- ❒ Using a fine pair of tweezers, pull out the lock washer and take the filter out.
- ❒ Place new filter into the filter wheel such that the colored side is facing up and the metallized side is facing down. If the side of the filter contains an arrow mark, the arrow has to point up (towards the light).
- ❒ Push in lock washer to fix the filter.
- ❒ In the **Emission Filter Wheel** dialog box, drag the filter onto the respective positions of the filter wheel name.

Cleaning filters

- ❒ Filters should be cleaned using a lint-free cloth or, better, a micro fiber cloth, as used for cleaning eye glasses.

- ❒ Insert filter wheel again. It can only be inserted in a certain position: the metal pin of the filter wheel has to be fitted into the hole in the base plate, without twisting this hole, to keep the original change position. The filter wheel is inserted correctly when it rests flat on the base plate.
- ❒ Put cover plate on filter wheel and make sure the round countersinks are flush with the dowel pins. Then turn the cover plate slightly clockwise, so that the countersink in the center of the cover plate is exactly facing the back (see Figure 3-5).
- ❒ Hold photomultiplier, pull locking button (left) and carefully tilt photomultiplier down again. It has to be inserted directly into in the respective opening. Check as follows: it must be possible to push the PMT with the cover plate easily to the mobile reading positions. For a certain time after filter replacement, the photomultiplier may show increased background values.
- ❒ Close instrument cover again and fix it with screws.

3.2.6 Injectors

The injectors (optional) are located inside the instrument on the rear panel. The injector ports are directly accessible to the operator. The tubings from the solution bottles are connected to the injector ports using screw-type caps. The instrument rear panel includes a device for installation of the supply bottles („reagent bottle backpack“).

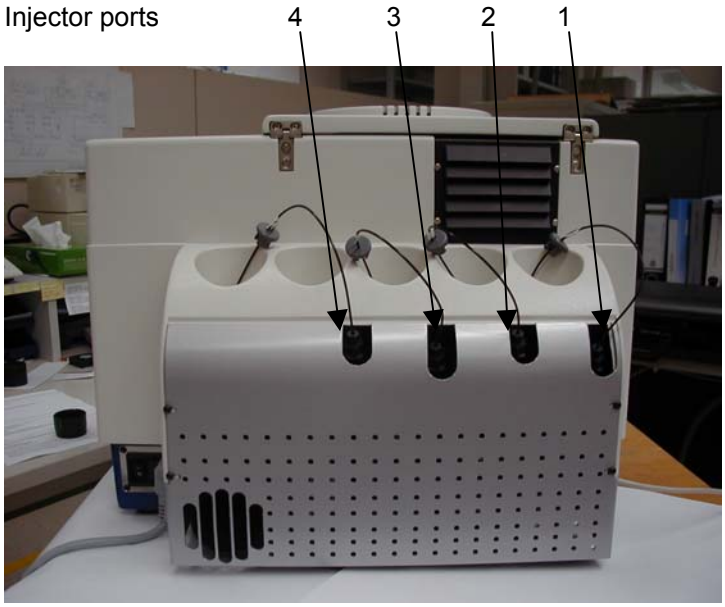


Figure 3-6: Mithras rear panel with reagent bottles „back pack“

Four injector positions inside the reader unit

J1	pre-position luminescence
J2 and J3	reading position luminescence
J4	reading position fluorescence

Injector configuration

Injector 1 Luminescence: injection into the well located 1 position before the reading position.

Injector 2 + 3 Luminescence: injection into the well in reading position.

Injector 4 Fluorescence: top reading position.
Injector 4 will be discussed in a separate section, for technical reasons: J4 may only be washed and primed if a prime plate has been inserted, as there is no aspiration pump installed at this point!!!

Injector parameters

The parameters for control of the injector are entered into the software:

- ❒ For measurement in menu item **Options | Read**,
- ❒ For washing and priming on the **Instrument** menu.

3.2.7 Excitation Halogen Lamp with Fan

The fan is located on the right instrument side and behind it, inside the instrument, the excitation halogen lamp (0 – 75 W).
The fan works in continuous service when the instrument is turned on. Mithras works correctly only if the fan is working properly. If the fan is faulty, the instrument has to be turned off to rule out overheating and the fan has to be replaced.
The excitation halogen lamp can be set via software in 65 535 digital steps between 0 and 75 Watt (see also chapter 5.5.5).

Figure 3-7: Right instrument side: fan and excitation halogen lamp

If the excitation halogen lamp is faulty, first screw off the fan and take it off. Then pull the halogen lamp carefully out and remove it.

3.2.8 Connections

Connections (PC, power supply), mains switch and instrument fuse are located on the instrument rear panel to the left next to the „reagent bottles backpack“. The reagent outlet is located on the right instrument side.

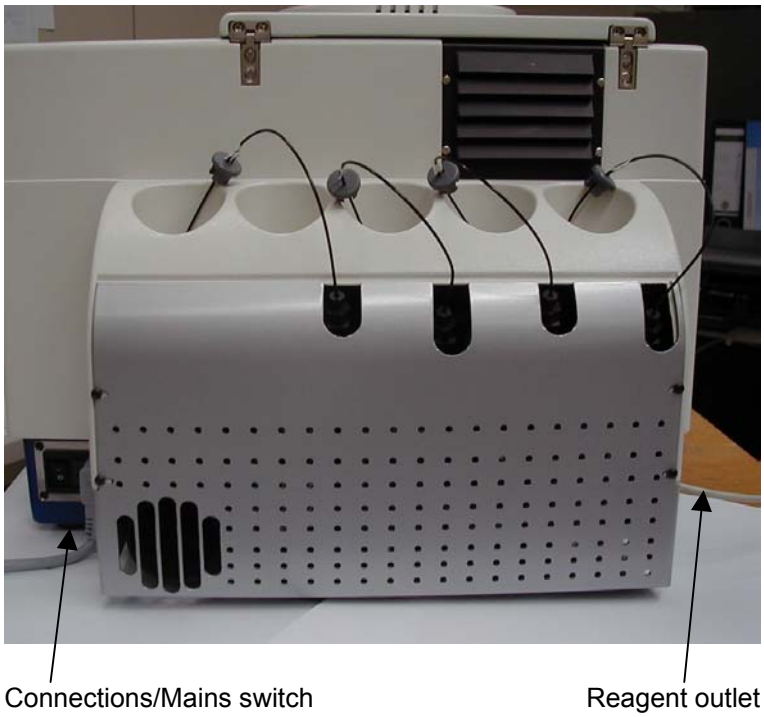


Figure 3-8: Connections and mains switch on instrument rear panel

Serial port
Connect a computer (PC, laptop) for control of the ***LB 940 Mithras*** to the 9-pole serial port.

Mains plug
Connect the instrument to mains using the cable supplied with the instrument (with grounded conductor).

Mains switch
Push the mains switch to turn the instrument on and off. Turn the instrument on before starting the software, so the program can establish communication with the instrument. If the instrument is turned on after you have started the software, you have to select **Instrument | Boot Instrument** to establish communication with the instrument.

Reagent outlet
If a waste pump is installed, connect a waste tubing to it and put this tubing into a waste bottle.
Observe all statutory requirements for handling patient samples and reagents!

Fuse
The instrument fuse is located next to the mains switch in a black fuse holder. For fuse replacement please see chapter 6 Maintenance.

3.2.9 PC

Requirements

Pentium processor, 500 MHz (or better)
128 MB RAM
Graphics card 1024x768 (or better)
CD-ROM drive
Operating system: Win98, Win2000, Win NT, Win XP
Serial port RS232
Parallel port

3.3 Software

3.3.1 Structure

The software for control of the Multilabel Reader ***Mithras*** is an extension of the *Windows* application ***MikroWin2000***.
The illustration below shows the menus and functions of the program. Only the system functions printed in boldface type are described in this user guide. For information on all other functions please refer to the user guide and the ***MikroWin2000*** online help.

File	Edit	View	Read	Instrument	Options	Installation
New	Undo	Tool Bar		**Load Plate**	**Read**	Settings
Open	Copy	Status Bar		**Unload Plate**	Threshold	Automation
Save	Paste	Navigation Bar		**Prime**	Controls	**Driver**
Save As	Reset	Help Card Bar		**Wash**	Definitions	
Export				**Refresh**	Matrix	
Export Setup				**Unload Injector**	Result	
Print				**Excit. Filter Slide**	Curve Fit	
Print Preview				**Emiss. Filter Wheel**	Kinetics	
Print Setup				**Boot Instrument**	Scanning	

3.3.2 Brief Explanation of Menus and Functions

File menu

To open, save, export or print files and enter the respective parameters.
Select the desired export driver for the active parameter file via the **Export** item.
Enable an automatic export function for the active parameter file via **Export Setup**. To do this, select the desired export driver. It must be the same as the driver selected in the menu item **File | Export**. If this function is enabled, data is exported automatically upon completion of a measurement. See also chapter 5.3.

View menu

To select special views. For example you can show or hide the navigation bar or the tool bar.

Read menu

To start a measurement using the active parameter file.

Instrument menu Instrument functions are presented on this menu:

Load Plate	To move the plate slide into the instrument below the photomultiplier.
Unload Plate	To move the plate slide out of the instrument to unload a microplate and load a new one.
Prime	To prime the injector tubings. Select the injectors and the number of shots and then start the prime function. Please don't forget to load a prime plate if no waste pump is installed in the instrument; you always have to load a prime plate for injector 4. The instrument detects this and prompts you accordingly.
Wash	To wash the injector tubings. Select the injectors and the number of shots to wash the tubings. Don't forget to load a prime plate if no waste pump is installed in the instrument; you always have to load a prime plate for injector 4. The instrument detects this and prompts you accordingly.
Refresh	To refresh tubings with one shot. Please keep in mind that you have to load a prime plate if no waste pump is installed in the instrument; you always have to load a prime plate for injector 4. The instrument detects this and prompts you accordingly.
Unload Injector	To pump any solution remaining in the tubings back to the bottle.
Excitation Filter Slide	To move the excitation filter slide out/in (instrument front panel) and to define the filter used.
Emission Filter Wheel	To change and to define the emission filter.
Boot Instrument	To establish communication between instrument and PC if the ***Mithras*** instrument has been turned on an off again while working with ***MikroWin2000***.

Options menu To define the reading parameters.
Select **Read** to open the **Options Read** dialog box to define the wells to be read and the reading sequence including individual operations.

Installation menu To define the settings (directories, password, etc.) in the **Installation Settings** dialog box.
To select and define the drivers for the ***Mithras*** instrument and for the export function in the **Installation Driver** dialog.

4. Getting Started and First Measurement

4.1 Setup Site

The Multilabel Reader ***Mithras LB 940*** has to be set up in dry, fairly dust-free rooms and protected from exposure to direct sunlight and significant temperature fluctuations. It should not be set up next to a radiator or an air conditioning.

4.2 Space Required

Dimensions of LB 940: 330 x 430 x 270 mm (W x D x H)

Set the instrument up such that the rear panel with the connection ports is easily accessible, so it can be turned on and off easily any time.

Allow for sufficient space on the side for the waste and liquid bottles (reagents, wash solution).

Do not set up the ***Mithras*** instrument close to the PC.

4.3 Unpacking

The cardboard box is reusable and should be used again whenever the instrument has to be transported.
When unpacking the instrument, make sure the shipment is complete and shows no sign of damage. The careful packing usually rules out transport damages. Should the instrument or instrument parts be damaged, anyway, please inform the shipping agent or the BERTHOLD TECHNOLOGIES service department immediately!

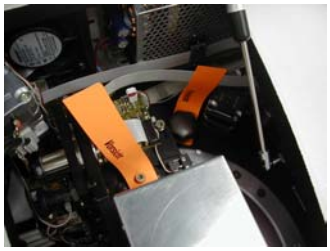
Unpack instrument and remove foamed inserts with care!

4.4 Connecting

Check if power supply is within the permissible range of the Mithras's operating voltage. Connect instrument only if this is the case!

Remove transport safety screw:

❒ Carefully take instrument out of cardboard box and put it onto your workplace.



(a)

(b)

Remove transport safety screw

❒ Two red tapes remind you to remove the transport safety screw (a).

❒ Unscrew instrument cover and open it. The signal tapes indicate the position of both screws (a).

❒ Unscrew transport safety screw 1 using an Allan key. It fixes the photomultiplier to the guide rail, so it cannot move during transport and is not damaged (b).

❒ Keep transport safety screw in a safe place. It has to be reused when transporting the instrument.

❒ Remove second transport safety screw and also keep it in a safe place (c).

❒ Close instrument cover and fix it again with screws..

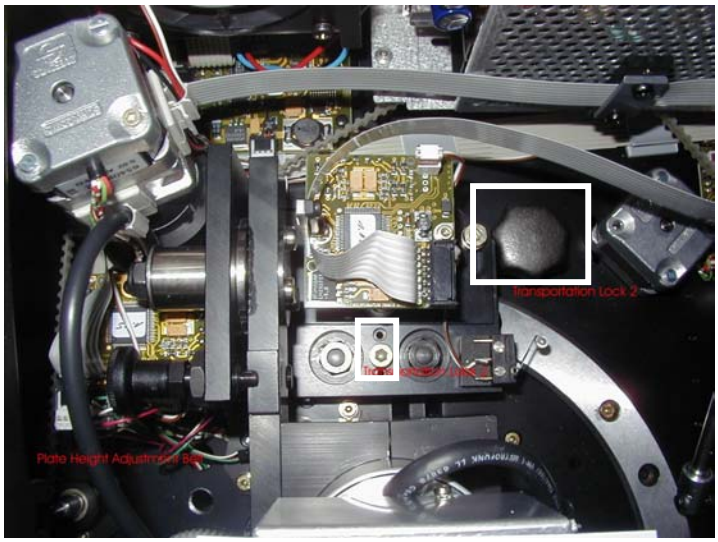


Figure 4-1: LB 940 – inside view

Install both transport safety screws again before transporting the instrument! Please read chapter 6.4 Preparations for Transport .

Electrical connections

- ❒ Connect hardlock supplied with the instrument to the parallel port of your PC.
- ❒ Connect ***Mithras*** instrument (PC port) to serial port of PC or laptop.
- ❒ Connect instrument to the wall outlet using the power cord supplied with the instrument.
- ❒ If you have not already done so, connect the computer to the wall outlet.

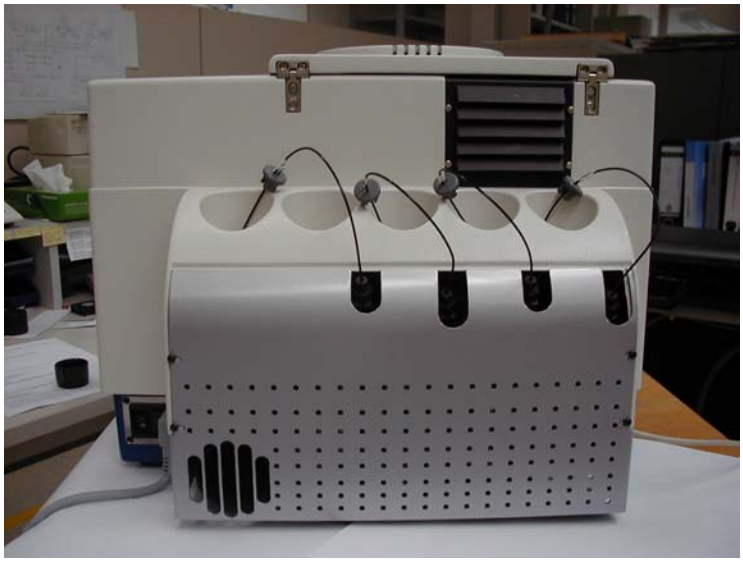


Figure 4-2: Mithras rear panel with connections

Waste and reagent tubings

- ❒ If the instrument includes a *waste pump,* connect tubing supplied to the reagent outlet.
- ❒ Pass waste tubing into a suitable bottle to collect the wash solution.
- ❒ Connect reagent bottle. Fix reagent tubing to the injectors with screws. The injectors are located on the instrument rear panel („Reagent bottles backpack“) and are numbered as follows (from right to left):

Injector 1 Luminescence: injection into the well located on position before the reading position.

Injector 2 + 3 Luminescence: injection into the well in reading position.

Injector 4 Fluorescence: Top reading position.

For technical reasons injector 4 will be discussed in a separate section: J4 may only be washed and primed if a prime plate has been inserted, as there is no aspiration pump installed at this point!!!

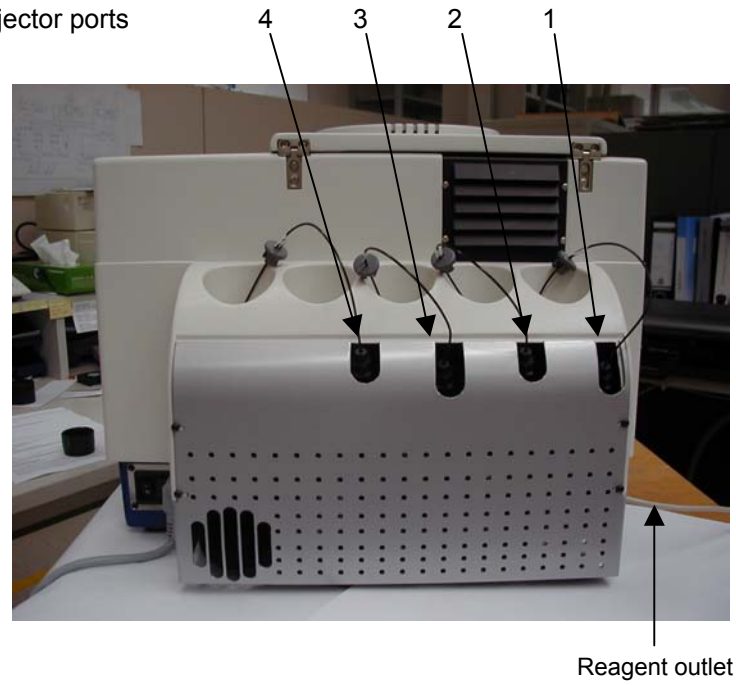


Figure 4-3: Injector ports on rear panel

Power on

- ❒ Push mains switch (on instrument rear panel) to turn instrument on.

4.5 Software Installation

- ❒ Install ***MikroWin2000*** software on your computer (see chapter 2.2.1)
- ❒ Install driver software (see chapter 2.2.2) and select the driver for the Mithras instrument.
- ❒ Define excitation and emission filters on the **Instrument** menu and install them in the instrument accordingly (see chapter 5.4.8 and 5.4.9).

4.6 First Measurement

4.6.1 Luminescence Measurement without Injections

- ❒ Start ***MikroWin2000*** software.
- ❒ Select **File | Open**.
- ❒ Select file type ***par** in the **Open** dialog box.
- ❒ Open basic parameter file **Lumi_01s.par**.
- ❒ Click <**Read**> button in the main window.
- ❒ Enter a file name for the data in the status bar. The file name of the parameter file with the extension *.dat is defaulted.
- ❒ Click the <**Start**> button. The plate slide moves out and on the screen you see the prompt to load a microplate.
- ❒ Place a prepared microplate onto the plate slide; make sure A1 is located in the rear left corner.
- ❒ Click <**OK**> to confirm the prompt. The plate moves into the instrument below the photomultiplier and the selected wells are read.
- ❒ Select the **Results** dialog box to view the results on the matrices.
- ❒ Results may be displayed graphically, printed out or exported (e.g. as an Excel file).

4.6.2 Luminescence Measurement with one Injection

Example: Luminescence measurement, injection in reading position

- ❒ Connect respective reagent bottle to injector 3.
- ❒ Start ***MikroWin2000*** software.
- ❒ Select **File | Open**.
- ❒ Select file type ***par** in the **Open** dialog box.
- ❒ Open basic parameter file **Lumi1s.par** for a luminescence measurement with one injection (Inj. 3) in reading position. All 96 wells have been pre-selected for reading and injection.
- ❒ Select **Instrument | Prime**. The **Injector Sequence** dialog box is displayed.
- ❒ Select injector 3.
- ❒ If no waste pump is connected, you have to place a prime plate onto the plate tray. In this case, the plate tray opens. Insert the prime plate and click <**Next**>. The plate tray is closed and the prime plate is placed below the injectors.
- ❒ Connect the reagents to the respective injectors.
- ❒ Click <**Next**> as soon as you have connected the reagents correctly.
- ❒ Then the selected injector tubing is filled with 12 shots. A corresponding message is displayed in the **Injector Sequence** dialog box. Wait until the prime process is finished.
- ❒ As soon as the prime process is finished, a corresponding message is displayed and the <**Close**> button is enabled. Click on <**Close**> to complete the process and to close the **Injector Sequence** dialog box.
- ❒ If you are working with a prime plate, this plate is automatically moved out as soon as you click <**Close**>. Remove the prime plate and close the plate tray again by selecting **Instrument | Load Plate**.
- ❒ As soon as the tubing is primed, click the <**Read**> button in the main window.
- ❒ Enter a file name for the data in the status bar. The file name of the parameter file with the extension *.dat is defaulted.
- ❒ Click the <**Start**> button. The plate slide moves out and you are prompted to load a microplate.
- ❒ Insert a prepared microplate onto the plate slide such that A1 is located in the rear left corner.
- ❒ Click <**OK**> to confirm the prompt. Then the plate moves into the instrument below the photomultiplier and the selected wells are read.
- ❒ Select the **Results** dialog box to view the results on the matrices.
- ❒ Results may be displayed graphically, printed out or exported (e.g. as an Excel file).

4.6.3 Fluorescence Measurement without Injections

- ❒ Start ***MikroWin2000*** software.
- ❒ Select **File | Open**.
- ❒ Select file type ***par** in the **Open** dialog box.
- ❒ Open the basic parameter file **Fluoreszein.par**.
- ❒ Click the <**Read**> button in the main window.
- ❒ Enter a file name for the data in the status bar. The file name of the parameter file with the extension *.dat is defaulted.
- ❒ Click the <**Start**> button. The plate slide moves out and on the screen you see the prompt to load a microplate.
- ❒ Place a prepared microplate onto the plate slide; make sure A1 is located in the rear left corner.
- ❒ Click <**OK**> to confirm the prompt. The plate moves into the instrument below the photomultiplier and the selected wells are read.
- ❒ Select the **Results** dialog box to view the results on the matrices.
- ❒ Results may be displayed graphically, printed out or exported (e.g. as an Excel file).

5. Mithras Software Functions

5.1 Software Structure and Operation

In the following section you find information on the structure of the software. For more detailed information please consult the MikroWin2000 software online help.

Program start

Double-click on the **program icon** to start the software. The main window is displayed.

Main window

Upon program start, this main window includes six **child windows**, i.e. **Data**, **Template**, **Calculation**, **Results**, **Statistics** and **Graphics**. You can work with these windows in accordance with the ***Windows Multi Document Port*** (MDI) definition.
In addition, the **navigation bar** can be displayed; from this navigation bar you can select the **child windows** with the respective views.

Menu bar

All program functions can be selected from the **menu bar**.

Tool bar

The **tool bar** of the main window is located directly below the menu bar, providing quick access to various windows and to the print, edit and file operations simply by clicking on the respective tool button.

Status bar

The program status bar is located at the bottom of the main window. It contains information on the currently loaded data and parameter file. Context-sensitive help information is available in the left section of the status bar. The name of the active parameter file (*.par) is displayed in the right-hand section.

Result file

The raw data of the measurement is saved to a **result file** (extension *.dat). Furthermore, this file may contain the respective sample identifier for each cavity. Any errors that may occur during measurement are also stored. In addition, the result file includes the data from the child windows **Data**, **Results**, **Statistics** and **Graphics** as well as further information, such as date and time of measurement and the parameter file used.

Parameter file

The **parameter file** (extension *.par) includes all test-specific settings which are required to run and evaluate tests, including all measurement parameters (well selection, measurement sequence, filter selection, etc.), all calculation parameters (data from the child windows **Template** and **Calculation**) as well as all print and export settings.

Basic parameter files

The basic parameter files supplied by ***Berthold Technologies*** include typical measurement sequences of some major types of measurement. Using this data, you just have to make a few changes to define the measurement sequence for your own requirements. A parameter file includes selected wells, predefined operations and evaluation parameters (e.g. calculations, result presentation and export). The file name indicates the respective type of measurement:

Bret1.par	BRET: 2 measurements without injections (Coelenterazin & eYFP)
Bret2.par	BRET: 2 measurements without injections (Deep Blue C & GFP²)
Dlr.par	Dual Luciferase Reporter Gene Assays with 2 injections
Dlr_0.par	Dual Luciferase Reporter Gene Assays without injections
Dlr_384.par	Dual Luciferase Reporter Gene Assays in 384 well plate
Fluorescein Kin1.par	Fluorescence kinetics measurement with injector 4
Fluorescein Rep10.par	Fluorescence repeated measurement with injector 4
Fluorescein.par	Fluorescence measurement without injection
Lumi 1s Kin10.par	Luminescence kinetics measurement with injector 3
Lumi 1s Rep10.par	Luminescence repeated measurement with injector 3
Lumi 1s.par	Luminescence measurement with injector 3
Lumi_0 1s.par	Luminescence measurement without injection
Readit.par	Promega Readit™ measurements
Umbelliferone.par	Fluorescence measurement without injection

Save the changes to these files under a *new name* to keep the basic parameter files!

Matrices

The matrix system of the program is largely comparable to a spreadsheet software. However, the number of input fields is limited to 8 x 12 positions, representing the typical microplate format. Plates with less or more cavities may also be emulated. Using this matrix system, all calculations which are required to evaluate a test can be carried out.

Operation

Operation and handling of the program follow the usual *Windows* conventions. Special user instructions will be described whenever required.

The following sections of chapter 5 describe only those functions in detail which are relevant for control of the ***Mithras*** instrument. In addition, we will discuss some menu items which are essential for creating parameter files and result output. These functions are printed in boldface type in the menu overview below.

Menu overview

File	Edit	View	Read	Instrument	Options	Installation
New	Undo	Tool Bar		**Load Plate**	**Read**	Settings
Open	Copy	Status Bar		**Unload Plate**	Threshold	Automation
Save	Paste	Navigation Bar		**Prime**	Controls	**Driver**
Save As	Reset	Help Card Bar		**Wash**	Definitions	
Export				**Refresh**	Matrix	
Export Setup				**Unload Injector**	Result	
Print				**Excit. Filter Slide**	Curve Fit	
Print Preview				**Emiss. Filter Wheel**	Kinetics	
Print Setup				**Boot Instrument**	Scanning	

Menu items printed in boldface type are described in the following chapters:

		Chapter
File | Open	Open parameter files	**5.5.2**
	Open data files	**5.6**
File | Save (As)	Save parameter files	**5.5.2**
	Save data files	**5.6**
File | Export (Setup)	Export data files	**5.3**
Read	Measurement	**5.6**
Instrument | ...	Instrument control functions	**5.4**
Options | Read	Definition of measurement sequence	**5.5.3**, **5.5.4** and **5.5.5**
Installation | Driver	Driver setup	**5.2.2**

5.2 Installation

5.2.1 MikroWin2000 Installation

(see Quick Reference Guide page 8)

5.2.2 Driver Installation

(see Quick Reference Guide page 10)

5.2.3 Driver Setup

In order to control the Multilabel Reader ***Mithras*** via the software, you have to set up the respective drivers. To work with ***Mithras*** you have to set up the **Reader** and **Export** drivers correctly. Select the menu item **Installation | Driver** to open the **Installation Driver** dialog box with a separate tab for each driver type.

Neues Bild mit englischer Software!

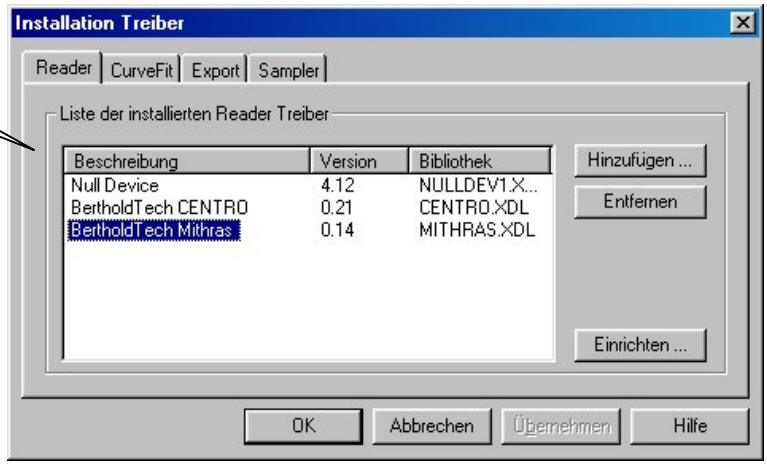


Figure 5-1: ***Installation driver*** *dialog box*

Installation Driver dialog box

List of installed drivers

This list includes all currently installed drivers of a special type (**Reader**, **CurveFit**, **Export** and **Sampler**). Each driver type appears on a separate tab.
The left column shows the names of the installed drivers; the center column shows the version number of the driver and the right column includes the names of the library file used for execution of the driver functions.

<Add Driver>

Click this button to add a new driver or to update an existing driver. You need a program library, i.e. a file with the extension xdl (e.g. Nulldev1.xdl). Such a file may be found on a special driver disk, a program CD, the local hard-drive or on the network. Click the <**Add**> button to open a dialog box from which you can select the drive and directory of the new library file. Click the <**Browse...**> button to search for a special folder. If the directory of the library file has been defined, click <**OK**> to open another dialog, listing all drivers (library files) available in the selected directory. Select one or several entries from the list and click <**OK**> to install the selected drivers.

<Delete Driver >

To delete an installed driver, you first have to select one from the list of installed drivers. Click the <**Delete Driver**> button to remove the selected driver.

<Driver Setup>

Click the <**Driver Setup**> button to configure an installed driver. Each new driver should be set up prior to its first use. A driver-specific dialog opens in which you can define the required configuration. The **Reader** driver dialog typically includes the com-port settings used as well as information on the injectors installed in the instrument, etc. This information should be verified after first installation. A general description of these dialogs is not possible since each driver has its own configuration dialog.

Mithras Driver Setup

- ❒ In the **Installation Driver** dialog box, click on the **Reader** tab and select the instrument driver **BertholdTech Mithras**.
- ❒ Then click <**Driver Setup**> to open the dialog box **BertholdTech Mithras** (with version number).

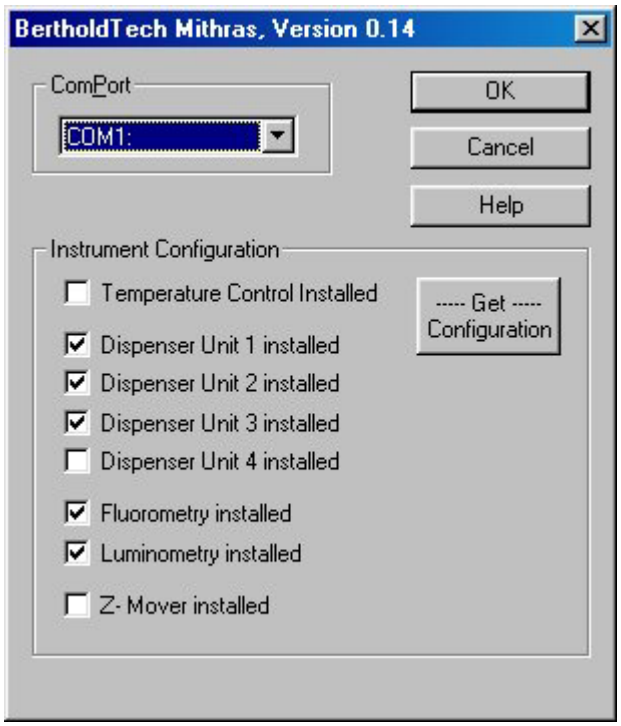


Figure 5-2: Setup of device driver

ComPort
Define the PC port to which the ***Mithras*** instrument is connected. You may choose **COM1**, **COM2**, **COM3** and **COM4**.

Instrument Configuration
Define the configuration of your ***Mithras*** instrument. A selected item is identified by a checkmark (✓).

Temperature Control Installed
Select this item if your instrument includes a temperature control.

Dispenser Unit ... installed
Here you define which injector(s) is/are installed. This setting specifies how many injectors are controlled and are available in the software for definition of the measurement sequence.
Up to max. 4 injectors can be installed at the same time. Please note the position and function of the individual injectors.

Injector 1: Injection before reading position (luminescence)
Injector 2: Reading position (luminescence)
Injector 3: Reading position (luminescence)
Injector 4: Reading position (fluorescence) at top reading

Fluorometry installed
Select this item if the ***Mithras*** unit is equipped for fluorescence measurements. A selected item is identified by a checkmark (✓).

Luminometry installed
Select this item if the ***Mithras*** unit is equipped for luminescence measurements. A selected item is identified by a checkmark (✓).

Z-Mover installed
Select this item if the ***Mithras*** unit includes an automatic plate height adjustment mechanism for the microplates in Z-direction (vertical). A selected item is identified by a checkmark (✓).

<**Get Configuration**> Download configuration from instrument.

<**OK**> Save configuration.

<**Cancel**> Discard new entries.

Export Driver Setup

Export drivers have to be installed if you want to export data. In addition, you have to set up the export driver and you have to specify data structure, data matrices as well as header and footer. Data is exported depending on the driver selected and configured in this dialog box. To use another data format, you can select another driver before running a measurement or set up the selected driver new.

❒ In the **Installation Driver** dialog box, select the **Export** tab to view the available drivers. You may choose:

Neues Bild mit englischer Software!

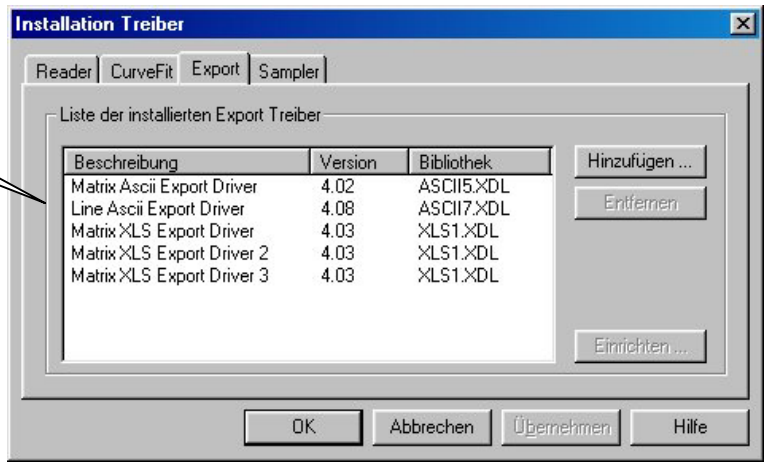


Figure 5-3: Selection of export drivers

Matrix Ascii Export Driver — Driver for data export to an Ascii file with matrix structure. The data is presented in the form of a matrix.

Line Ascii Export Driver — Driver for data export to an Ascii file with line structure. The data is presented row-wise.

Matrix XLS Export Driver — Driver for data export to an Excel file with matrix structure. Three XLS drivers are available; they can be set up for different purposes (e.g. different matrix numbers or header and footer).

❒ Select driver by clicking on the respective line.

❒ Click the <**Driver Setup**> button to open the respective dialog box and enter the parameters for data export.

Matrix XLS Export Driver If you select the Excel export driver, you have to define the following configuration:

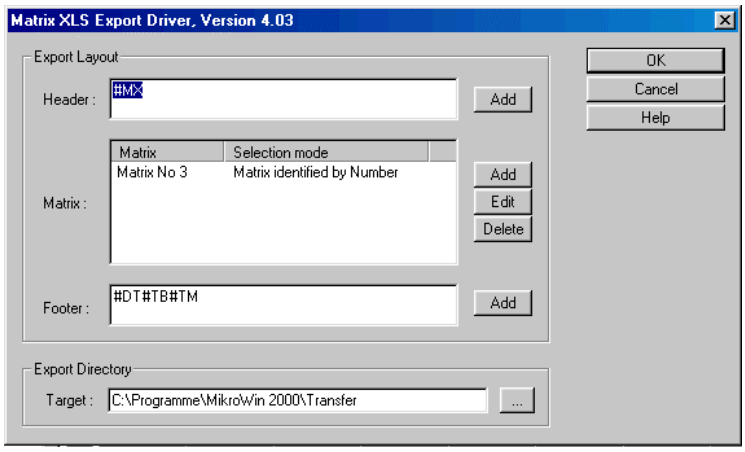


Figure 5-4: Excel export driver setup

Export Layout Define the Excel file layout in these three boxes.

Header Text box for entering a header. Click on the <**Add**> button to open a context menu and select a placeholder for the header. You may select several options one after another and separate the placeholders either by a tab character (**#TB**) or by a keyboard entry (comma, space, etc.).

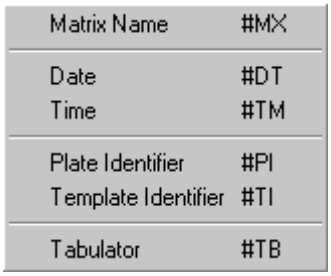


Figure 5-5: Context menu for entering header placeholders

Example:
Header with date, time and plate identification, separated by tab characters: **#DT#TB#TM#TB#PI**

Matrix

In this text box you enter the matrices whose data you wish to export. In general, one exports only data from the result matrices. But you may also select any other matrices with provisional results. ***Make sure*** that the matrix specified here is identical with the number of the result matrix in the parameter file. You should always use the same matrix number for the results in all parameter files. With respect to the supplied basic parameter files, the results are typically found on **Matrix 3**.
Click <**Add**> to open the context menu and select the matrix number (1 – 15) or define the matrix name. Several matrices can be selected one after the other. They are entered in the matrix list; the **Selection mode** column shows if the respective matrix is identified by its number or by its name. If the matrix is identified by its name, you have to enter the exact matrix name (e.g. **Results**). Click on the matrix name and then on <**Edit**>. The edit mode is enabled and you can enter the matrix name via the keyboard.
Click <**Delete**> to delete the selected matrix from the matrix list.

Footer

Text box for entering a footer. Click the <**Add**> button to open a context menu and select a placeholder for the footer. You may select several options one after another and separate the placeholders either by a tab character (**#TB**) or by a keyboard entry (comma, space, etc.). This context menu includes the same options as the header context menu.

Export Directory Target

Define the target directory for the file. The program proposes the subdirectory **Transfer** in the MikroWin2000 program directory. Click the <**...**> button to select another directory. The **Look in:** dialog box is displayed and you can select the desired directory.

Matrix Ascii Export Driver

Select this export driver to define an Ascii matrix file. Parameters (header, matrix and footer as well as the target directory for data storage) are entered in the same manner as for an Excel file (see previous section).
In addition, you have to specify the **Operation Mode** settings:

Operation Mode

Export only valid test
If this item is selected, data export takes place only if a test is valid.

Include matrix name title
If this item is selected, the matrix name is exported as well.

Line Ascii Export Driver

Select this export driver to define an Ascii file with row-wise data presentation.
Header and footer are defined by clicking the <**Add**>-button. The context menu includes the same options as the matrix export drivers.

Line

In this text box you describe the structure of the line. Click on the respective <**Add**> button to open the context menu showing the available options (see illustration to the left):

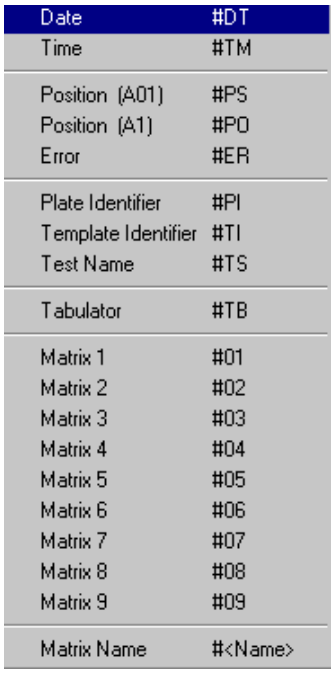


*Figure 5-6: Context menu **Line***

Position (A01)
Well position; numbers below 10 are preceded by 0.

Position (A1)
Well position without 0.

Error
Shows the error messages of the respective well.

Matrix 1...9
Selection of matrices whose data will be accepted.

Matrix Name
If this option is selected, you have to enter the exact matrix name, e.g. <**Results**> instead of <**Name**>.

Data Selection

In this group box you can select the data to be exported:

Consider Plate Partition
If this item is selected only the main well is exported if wells have been combined.

Skip Control Positions
If this item is selected only wells which are not used as controls will be exported.

Only used Sample ID's
Only the data of samples having an ID will be transferred.

Only numeric Sample ID's
Only the data of samples having a numeric ID will be transferred.

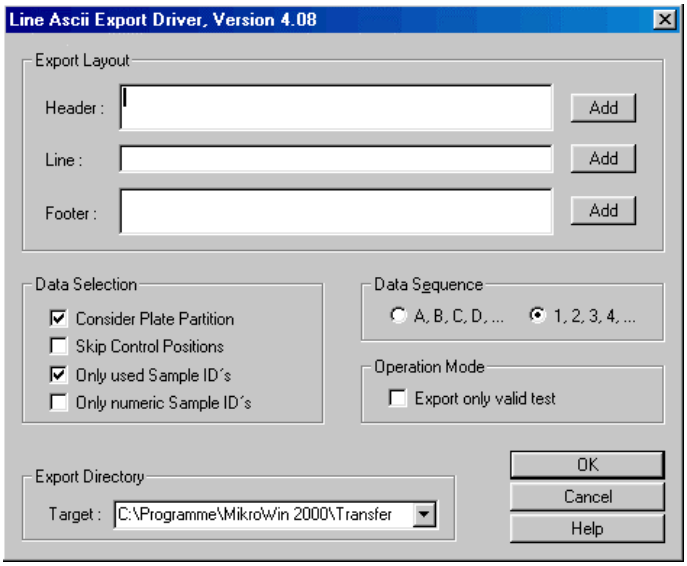


Figure 5-7: Parameters for a Line Ascii file

Data Sequence

In this group box you define the order of the data to be exported: **A, B, C, D...** means that the data in row A on the microplate will be exported, then those in row B, etc.
1, 2, 3, 4... means that the data in column 1 of the microplate will be exported, then those in column 2, etc.

Operation Mode

Export only valid test
If this item is checked, data export takes place only if a test is valid.

5.3 Export

Prerequisite for exporting data files is the correct installation and setup of the export driver (see chapter 5.2)

The following export functions apply only to the active parameter file and have to be saved with this file.

There are two different export functions:

a) **Manual data export** (**File | Export**): you select the export driver for the respective measurement. If you wish to export data after a measurement, click the <**Export**> button on the tool bar.

b) **Automatic data export** after each measurement. Prerequisite is that the export driver has been defined in the menu item **File | Export Setup**. After storing the parameter file, this setting is specific to this parameter file.

5.3.1 Manual Data Export

The following dialog supports manual export of program data. The data to be exported, the format as well as the export destination depend on the selected driver and its configuration. The actual data export is carried out by an export driver if you click on the <**Export**> button after a measurement.

❒ Open the parameter file you need.

❒ Select **File | Export** to open the **File Export** dialog box.

Neues Bild mit englischer Software!

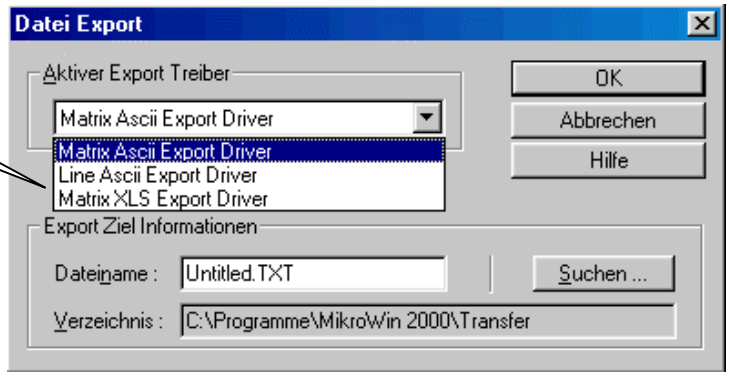


Figure 5-8: File Export dialog box with open driver list

Active Export Driver

Select the export driver you want to use for data export. Click on the arrow button to open the list showing the available drivers and select the driver you want. ***Please keep in mind*** that you have set up the driver you have selected here in the menu **Installation | Driver | Export**. Otherwise, no data will be transferred!

Export Target Information

File Name
Shows the file name of the active parameter file. An extension identifying the selected driver is appended (XLS for Excel files and TXT for Ascii files). The file name can be edited.

Directory
The target directory has been defined by the selected export driver during installation. Click the <**Browse**> button to select another target directory.

Click <**OK**> to accept your selection.

5.3.2 Automatic Data Export

Choose the menu item **File | Export Setup** to select the export driver that is to be loaded automatically upon successful completion of a reader run. If a driver has been selected for the active parameter file, data evaluation is performed after completion of the respective measurement and data export is carried out in accordance with the selected driver.

Please keep in mind:
This function is only valid for the active parameter file.
Prerequisite for automatic data export is that the respective export driver has been installed and set up in the menu item **Installation | Driver** (see chapter 5.1) and the export driver has been selected in the menu item **File | Export** (see chapter 5.2.1).

- ❒ Open parameter file.
- ❒ Select **File | Export Setup** to open the **File Export Setup** dialog box.

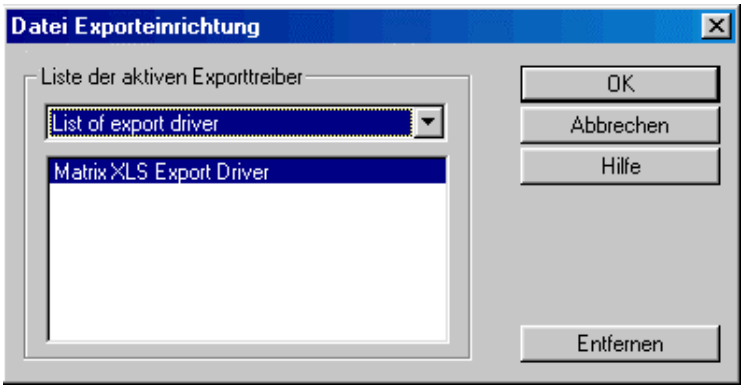


Figure 5-9: File Export Setup dialog box

List of Active Export Driver

Select the export driver you want to use for automatic data export upon successful completion of a reader run. Click on the arrow button to open the list showing the available drivers and select the driver you want. The selected drivers appear in the text box directly below the drop-down list box.
To delete a driver from the list, select this driver and then click <**Remove**>.

Click <**OK**> to accept your selection.

5.4 Instrument Control and Operation

The ***Mithras*** Multilabel Reader is controlled and operated via the ***MikroWin 2000*** software. This includes opening and closing of the plate tray as well as priming and washing the injector tubings.

Injector tubings have to be washed

- ❒ before starting work
- ❒ before changing reagents
- ❒ at the end of each work session before turning off the instrument
- ❒ after longer periods of inactivity

Use solutions recommended by the kit manufacturer, e.g. distilled water, diluted alcohol, hypochlorite solution ...

Injector tubings have to be primed

- ❒ prior to each measurement using the respective reagents.

A prime plate has to be used for the functions Prime tubings, Wash tubings and Refresh if no waste pump is installed.

For injector 4, please keep in mind:
This tubing can be primed, washed or refreshed in single steps. A prime plate always has to be used since a possibly installed aspiration pump cannot reach this position for technical reasons.

5.4.1 Instrument | Load Plate

Select **Instrument | Load Plate** to transport the plate slide into the instrument and to close the plate tray. The instrument is closed light-tight.

Open the plate tray only to load microplates, so that as little light as possible may penetrate the counting chamber. If necessary, allow for a delay between plate loading and start of measurement (programmed in the measurement sequence), since white microplates in particular may be phosphorescent after having been exposed to daylight.

5.4.2 Instrument | Unload Plate

Select **Instrument | Unload Plate** to move the plate slide out of the instrument and to unload a microplate.

Leave plate tray open only for loading microplates.

5.4.3 Instrument | Prime

Injector tubings have to be washed whenever changing reagents (see chapter 5.4.4) and then primed with the new reagent to ensure that the desired volume is already injected with the first shot.

NOTE

Use a prime plate whenever you are working with the prime function, if no waste pump is installed!
A prime plate always has to be used for injector 4!

Proceed as follows

- ❒ Select **Instrument | Prime** to open the **Injector Sequence** dialog box (Figure 5-10).
- ❒ Select the injectors to be primed. All 3 injectors may be selected at the same time.

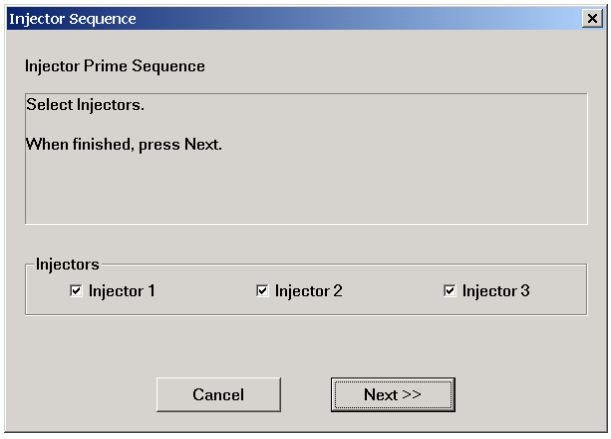


Figure 5-10: Select injectors

- ❒ If no waste pump is installed, you are prompted to load a prime plate. The plate slide moves out. Load the prime plate and click <**Next**>. The plate tray is closed and the prime plate is placed below the injectors (Figure 5-11).

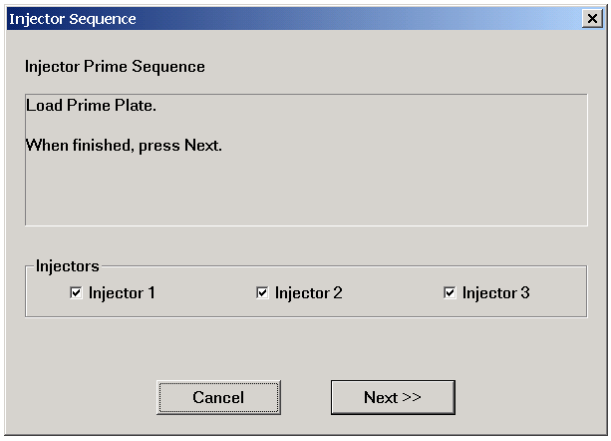


Figure 5-11: Load prime plate

- ❒ You are prompted to load reagents in the selected reagent positions (Figure 5-12).
- ❒ As soon as you have loaded the reagents correctly, click <**Next**>.

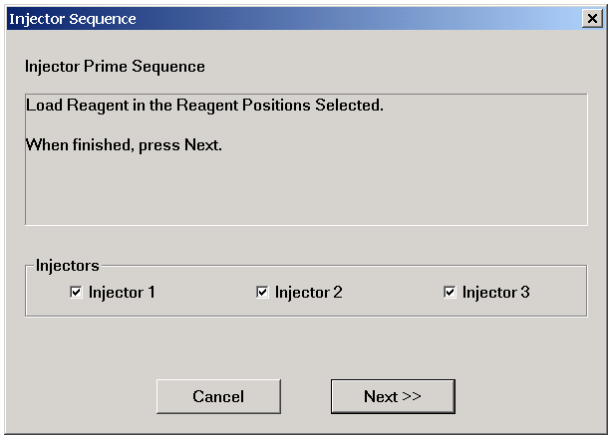


Figure 5-12: Connecting reagents to the injectors

- ❒ Then the selected injector tubings are primed with 12 shots. The **Injector Sequence** dialog box shows the respective information. Wait for the prime process to finish (Figure 5-13).

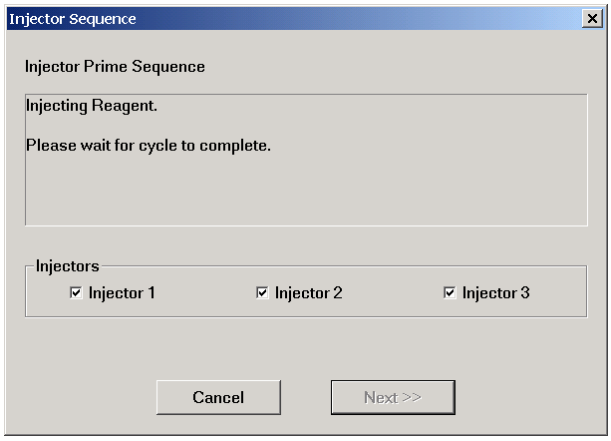


Figure 5-13: Prime injector tubings by 12 shots

- ❒ The end of the prime cycle is displayed on the screen and the <**Close**> button is enabled. Click <**Close**> to complete the process and to exit the **Injector Sequence** dialog box.
- ❒ If you are working with a prime plate, this plate is automatically moved out as soon as you click <**Close**>. Remove the prime plate (Figure 5-14).

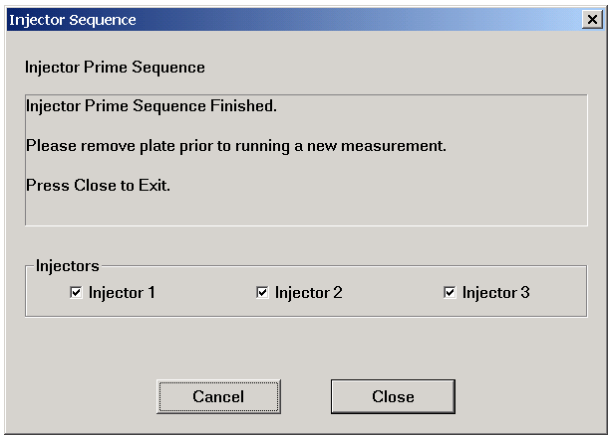


Figure 5-14: Finishing the prime process

5.4.4 Instrument | Wash

Injector tubings have to be washed whenever you are changing reagents as well as before starting and after completing your work.
Use the solutions recommended by the kit manufacturer.

NOTE

Use a prime plate whenever you are working with the wash function, if no waste pump is installed!
A prime plate always has to be used for injector 4!

Proceed as follows

- ❒ Select **Instrument | Wash** to open the **Injector Sequence** dialog box.
- ❒ Select the injectors you want to wash. All 3 injectors can be selected at the same time (Figure 5-15).
- ❒ Enter the number of wash cycles. One wash cycle consists of one shot. 10 cycles ensure that the injection system is washed completely.

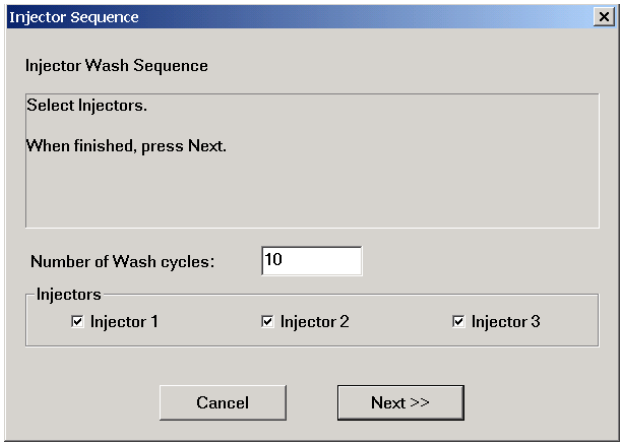


Figure 5-15: Selection of injectors and definition of the number of wash cycles

- ❒ If no waste pump is installed, you are prompted to place a prime plate into the plate tray. The plate slide moves out. Load the prime plate and click <**Next**>. The plate tray is closed and the prime plate is placed below the injector tips. ***Never carry out a wash cycle without prime plate!***

❒ Then you are prompted to load the wash solution in the selected reagent positions (Figure 5-16).

❒ Click <**Next**> as soon as you have connected the wash solutions correctly.

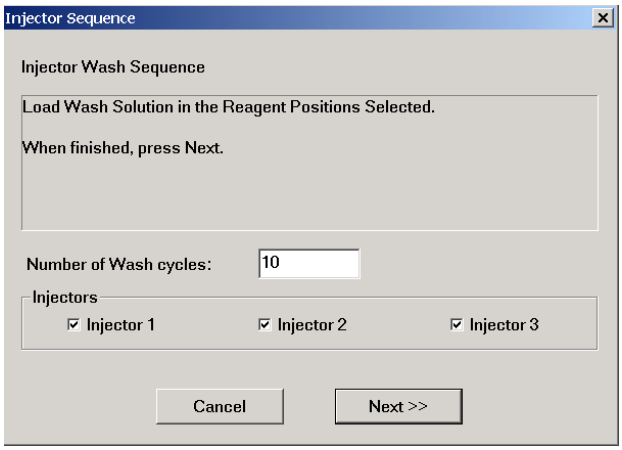


Figure 5-16: Loading the wash solution

❒ Then the selected injector tubings are washed using the number of wash cycles defined in this dialog box. The **Injector Sequence** dialog box displays a corresponding message. Wait until the process is finished (Figure 5-17).

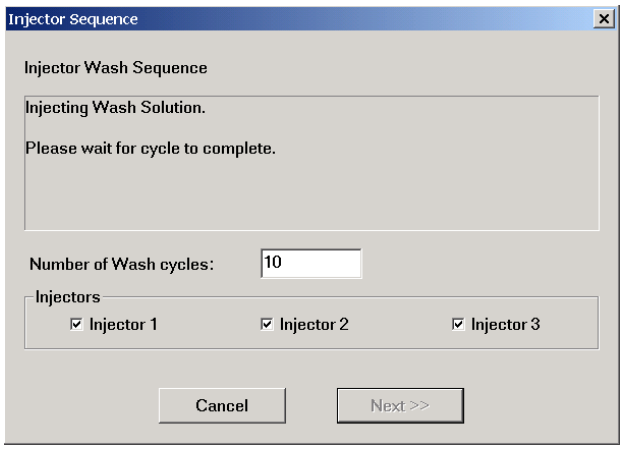


Figure 5-17: Washing the tubings

- ❒ The end of the wash cycle is displayed on the screen and the <**Close**> button is enabled. Click <**Close**> to complete the process and to exit the **Injector Sequence** dialog box.
- ❒ If you are working with a prime plate, this plate is automatically moved out as soon as you click <**Close**>. Remove the prime plate.

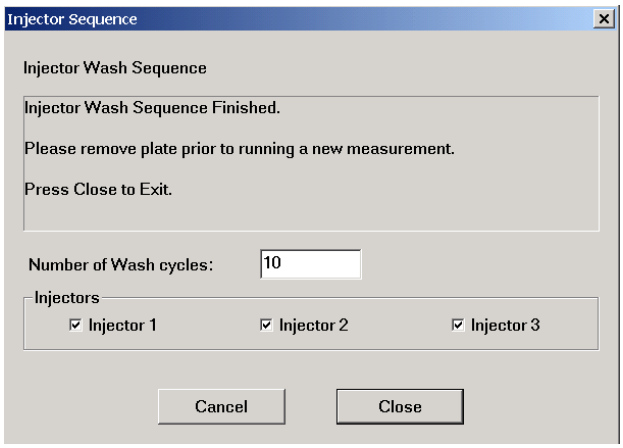


Figure 5-18: Completion of wash process

5.4.5 Instrument | Refresh

The function **Instrument | Refresh** may be used to refill reagent solution with one shot if the tubings have already been primed. This function is used, for example, when the last measurement took place some time ago and you are not sure if the tubing is completely primed.

NOTE

Use a prime plate whenever you are working with the refresh function, if no waste pump is installed!
A prime plate always has to be used for injector 4!

- ❒ Select **Instrument | Refresh** to open the **Injector Sequence** dialog box.
- ❒ Select the injectors for the refresh function.
- ❒ Click <**Next**>.
- ❒ The refresh function is carried out.

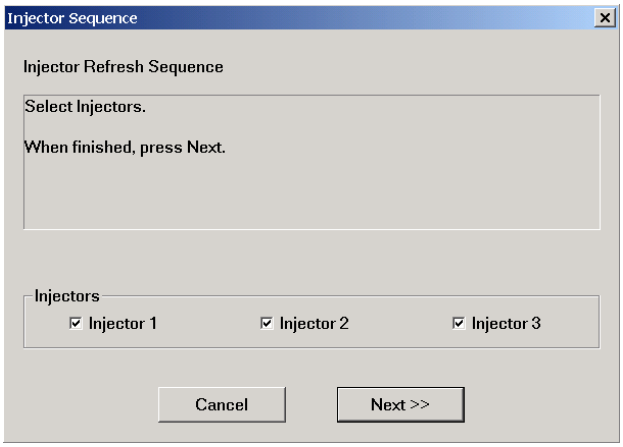


Figure 5-19: Refresh function

- ❒ If no waste pump is installed, you are prompted to place a prime plate into the plate tray. The plate slide moves out. Load the prime plate and click <**Next**>. The plate tray is closed and the prime plate is placed below the injectors.

5.4.6 Instrument | Unload Injector

Select **Instrument | Unload Injector** to pump reagents back into the bottle.

Make sure that bottles are connected to the respective injectors.

5.4.7 Instrument | Boot Instrument

If the ***Mithras*** instrument has been turned off and on again without having shut down the ***MikroWin2000*** program, you have to select the item **Instrument | Boot Instrument**. The software initializes the instrument again and establishes correct communication between instrument and PC. Otherwise instrument control is not possible.

5.4.8 Excitation Filter - Excitation Filter Slide

Definition and maintenance of the excitation filter used takes place via the menu item **Instrument | Excitation Filter Slide**. The following options are available:

- ❒ Enter the name and position of the filter used, which are then available for selection when defining the reading parameters (in the parameter files).
- ❒ Move the excitation filter slide in and out to change or clean the filter.

Select the menu item **Instrument | Excitation Filter Slide** to open the **Excitation Filter Slide** dialog box.

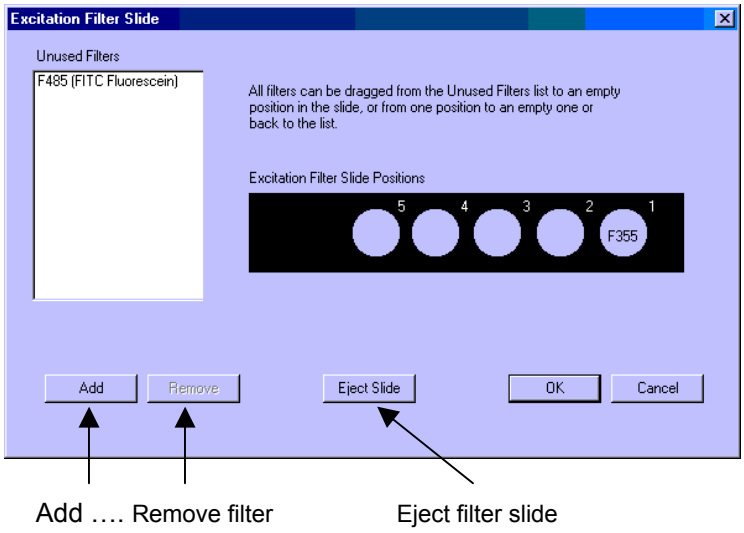


Figure 5-20: Control of excitation filter

Entering filter names

Click <**Add**> to open the **Add Filter** dialog box.

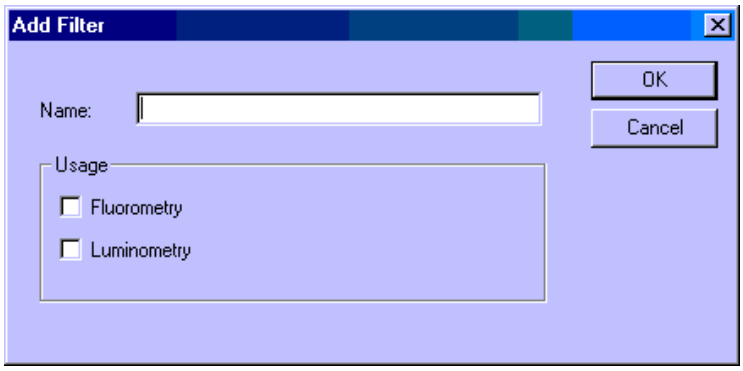


Figure 5-21: Entering a filter name

- ❒ Enter name of filter in the **Name** text box.
- ❒ Define usage of filter: for fluorescence or luminescence measurement.
- ❒ Click <**OK**> in the **Add Filter** dialog box. The dialog box is closed and the new filter name is listed in the **Unused Filter** text box in the **Excitation Filter Slide** dialog box.
- ❒ Delete a filter name: highlight the respective filter name and then click <**Remove**>.

Positioning filters

Depending on the position of the filter on the excitation filter slide, allocate the respective filter names to positions 1 to 5 (see Figure 5-22). In the parameter files only those filter positions are displayed for selection which are occupied here. Only those filters can be used for measurements which have been defined in the software. Therefore make sure that the allocation in the software matches the actual filter positions.

Proceed as follows

- ❒ Prerequisite is that filter names are displayed in the **Unused Filters** text box (see previous page).
- ❒ Drag[1] the filter name from the **Unused Filters** text box to one of the positions 1 to 5 of the schematic illustration of the excitation filter slide.
- ❒ You may also drag a filter name from one position on the slide to another, or from a slide position back to the **Unused Filters** text box.

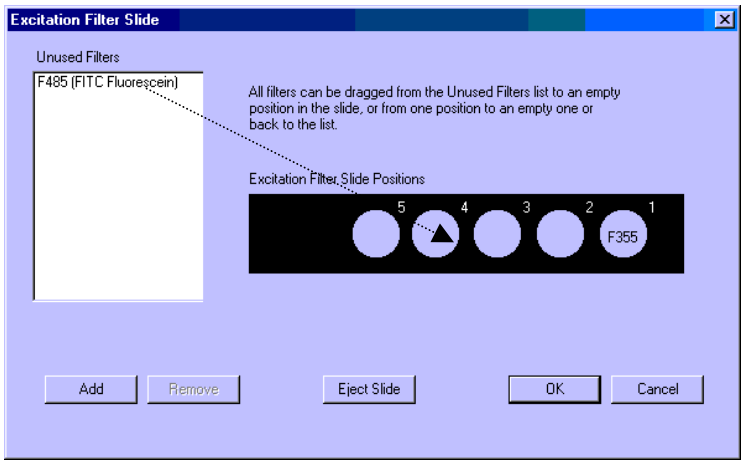


Figure 5-22: Placing filter names with drag & drop

[1] *Drag & Drop:* Place mouse pointer over filter name, push left mouse button and drag mouse pointer with mouse button held down to the desired position. The filter name is dragged along. Release left mouse button as soon as the filter name has reached the desired position. You can drop the filter name only in the **Unused Filters** box or on one of the 5 filter positions.

Replacing/Cleaning excitation filters

The compartment with the excitation filter slide is located to the right of the plate tray (instrument front panel).

Proceed as follows

- ❒ Click <**Eject Slide**> in the **Excitation Filter Slide** dialog box. The door opens slightly and the slide moves out a bit.
- ❒ Open the door all the way by hand and pull out the filter holder at both metal pins (bottom and top on filter holder).
- ❒ Clean filter or replace it and insert it again.
- ❒ If you replace the filter and insert it again, you have to drag the respective filter in the **Excitation Filter Slide** dialog box to the respective positions (see previous page).
- ❒ Push filter holder into the slide again all the way.
- ❒ Click <**OK**> in the **Excitation Filter Slide** dialog box. The slide moves into the instrument and the door is closed again.

5.4.9 Emission Filter – Emission Filter Wheel

The emission filter used is defined and serviced/managed using the menu item **Instrument | Emission Filter Wheel**. The following options are available:

- ❒ Enter the name and position of the filter placed in the filter wheel, which is then available for selection when defining the reading parameters (in the parameter files).
- ❒ Position the emission filter wheel to change or clean the filter.

Select the menu item **Instrument | Emission Filter Wheel** to open the **Emission Filter Wheel** dialog box. Drag the filters used to the desired filter wheel position.

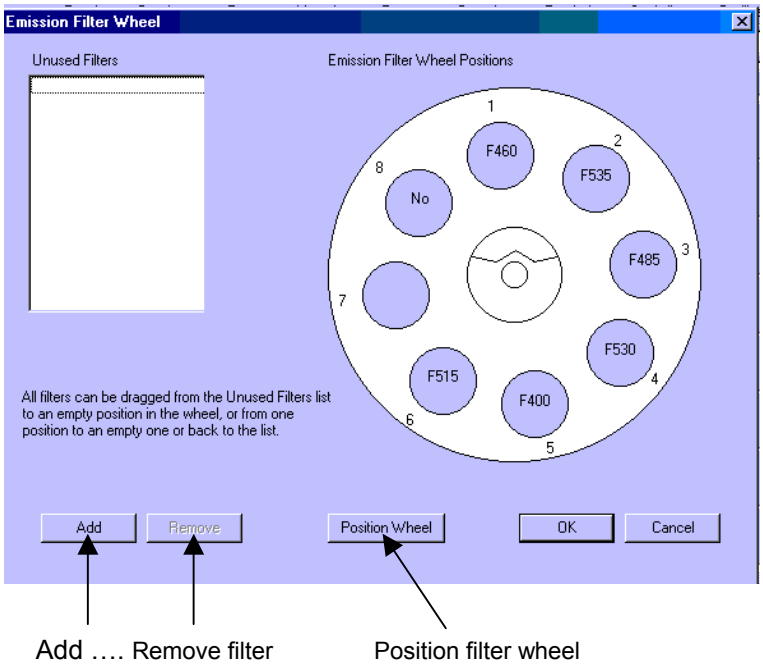


Figure 5-23: Emission Filter Wheel dialog box

Up to eight different emission filters can be placed into the emission filter wheel located directly below the photomultiplier. The desired filter is moved to the respective reading position (1, 2 or 3) under control of the software.

Entering filter names

- ❒ Click <**Add**> to open the **Add Filter** dialog box.
- ❒ Enter filter name in the **Name** text box.
- ❒ Define usage of filter: for fluorescence or luminescence measurement.
- ❒ Click <**OK**> in the **Add Filter** dialog box. The dialog box is closed and the new filter name is listed in the **Unused Filter** text box.
- ❒ Remove filter name: highlight the respective filter name and click <**Remove**>.

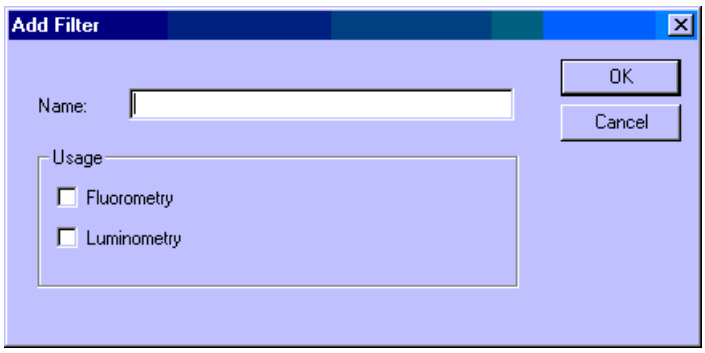


Figure 5-24: Entering a filter name

Positioning filters

Depending on the position of the filter in the filter wheel, allocate the respective filter names to positions 1 to 8 (see Figure 5-22). In the parameter files only those filter positions are displayed for selection which are occupied here. Only those filters can be used for measurements which have been defined in the software. Therefore make sure that the allocation in the software matches the actual filter positions.

Proceed as follows

- ❒ Prerequisite is that filter names are displayed in the **Unused Filters** text box.
- ❒ Drag the desired filter name from the **Unused Filters** text box to one of the positions 1 to 8 of the schematic illustration of the filter wheel.
- ❒ You may also drag a filter name from one position on the slide to another, or from a wheel position back to the **Unused Filters** text box.

Proceed as follows to replace the filter wheel

- ❒ Open instrument (open screw and raise cover).
- ❒ With the instrument turned on, select the menu item **Instrument | Emission Filter Wheel**. The **Emission Filter Wheel** dialog box appears showing a schematic presentation of the filter wheel.
- ❒ Click on the <**Position Wheel**> button. The filter wheel is turned to the replacement position. This position is extremely important and should not be twisted when taking out the wheel. The photomultiplier is located in reading position 1, all the way to the back.
- ❒ Pull off locking button (to the right of the photomultiplier) and raise the photomultiplier.
- ❒ Turn cover plate such that the 3 round countersinks are flush with the dowel pins, then take it off.
- ❒ Take out filter wheel and make sure that the base plate is not twisted.

Photomultiplier raised, cover plate accessible

Cover plate removed, filter wheel accessible

Replacing filters

- ❒ Using a fine pair of tweezers, pull out the lock washer and take the filter out.
- ❒ Place new filter into the filter wheel such that the colored side is facing up and the metallized side is facing down. If the side of the filter contains an arrow mark, the arrow has to point up (towards the light).
- ❒ Push in lock washer to fix the filter.
- ❒ In the **Emission Filter Wheel** dialog box, drag the filter onto the respective positions of the filter wheel name.

Cleaning filters

- ❒ Filters should be cleaned using a lint-free cloth or, better, a micro fiber cloth, as used for cleaning eye glasses.

- ❒ Insert filter wheel again. It can only be inserted in a certain position: The metal pin of the filter wheel has to be fitted into the hole in the base plate, without twisting this hole, to keep the original change position. The filter wheel is inserted correctly when it rests flat on the base plate.
- ❒ Put cover plate on filter wheel and make sure the round countersinks are flush with the dowel pins. Then turn the cover plate slightly clockwise, so that the countersink in the center of the cover plate is exactly facing the back.
- ❒ Hold photomultiplier, pull locking button (left) and carefully tilt photomultiplier down again. It has to be inserted directly into in the respective opening. Check as follows: it must be possible to push the PMT with the cover plate easily to the mobile reading positions. For a certain time after filter replacement, the photomultiplier may show increased background values.
- ❒ Close instrument cover again and fix it with screws.

5.5 Reading Parameters

5.5.1 Overview

The test-specific configuration required to perform and evaluate tests is saved to parameter files (extension *.par).
The **Mithras** Multilabel Reader can perform different types of luminescence and fluorescence measurements. Each type of measurement has its own typical sequence. ***Berthold Technologies*** supplies special basic parameter files, including typical measurement sequences, which can easily be adapted to each user's specific needs. You do not have to create a completely new measurement sequence, but you may utilize and modify an existing structure. The following basic parameter files are supplied:

Bret1.par	BRET: 2 measurements without injections (Coelenterazin & eYFP)
Bret2.par	BRET: 2 measurements without injections (Deep Blue C & GFP²)
Dlr.par	Dual Luciferase Reporter Gene Assays with 2 injections
Dlr_0.par	Dual Luciferase Reporter Gene Assays without injections
Dlr_384.par	Dual Luciferase Reporter Gene Assays in 384 well plate
Fluorescein Kin1.par	Fluorescence kinetics measurement with injector 4
Fluorescein Rep10.par	Fluorescence repeated measurement with injector 4
Fluorescein.par	Fluorescence measurement without injection
Lumi 1s Kin10.par	Luminescence kinetics measurement with injector 3
Lumi 1s Rep10.par	Luminescence repeated measurement with injector 3
Lumi 1s.par	Luminescence measurement with injector 3
Lumi_0 1s.par	Luminescence measurement without injection
Readit.par	Promega Readit™ measurements
Umbelliferone.par	Fluorescence measurement without injection

Save the changes to these files under a *new name* to keep the basic parameter files!

Reading parameters can be defined in two different ways:

a) Completely new definition of measurement and evaluation parameters.

b) Using an existing parameter file and modifying the parameters as needed. Either you use the basic parameter files supplied by ***Berthold Technologies***: each includes a typical measurement sequence for each type of measurement. Or you use parameter files you have created yourself and edit them.

5.5.2 Open/Save Parameter File

Create new parameter file

❒ Select **Edit | Reset** to create a new parameter file. The file name **Untitled.par** is displayed in the status bar.

Open parameter file

❒ Select **File | Open** or click the <**Open**> button on the tool bar to open an existing parameter file. The **Open** dialog box is displayed.

Neues Bild mit englischer Software!

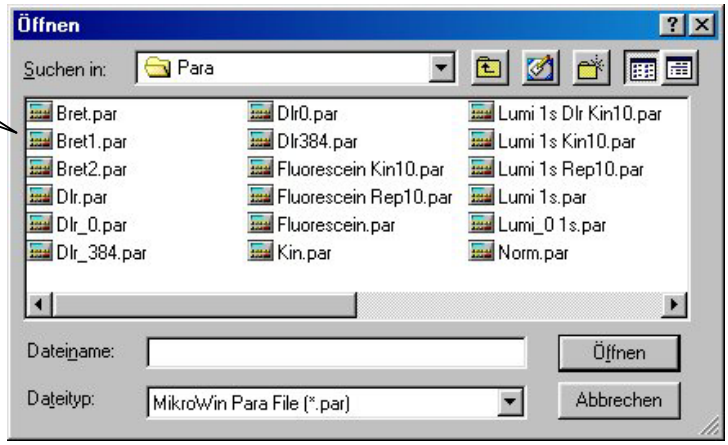


Figure 5-25: Open dialog box

❒ From the **Files of type** drop-down list box, select the type **MikroWin Para File (*.par)**. The contents of the directory **Para** is displayed, showing the available parameter files.

❒ Select the file you want and click <**Open**>.

❒ If the parameter file is located in another directory, open the **Look in** drop-down list box and select the directory you want.

The file name of the opened parameter appears on the status bar in the bottom right corner of the screen.

Please keep in mind:
All changes in the reading parameters refer to the open parameter file. Changes are taken over for good when the parameter file is saved.

Save parameter file

Select **File | Save** so save the active parameter file.

Select **File | Save As** if you want to save the file under a new name or if you have created a new parameter file („**Untitled.par**"). Enter the file name in the **Save As** dialog box and select the file type **MikroWin Para File** (extension *.par). The directory pre-defined in the **Installation settings** dialog box is defaulted. You can change the directory. Click <**OK**> and the new parameter file is saved to the pre-selected directory.

What does a parameter file contain?

A parameter file includes:

Well selection	**Options	Read**
Measurement sequence	**Options	Read**
Evaluation parameters	Child windows: **Data** **Template** **Calculation**
Sample parameters	**Options** menu Parameter for controls, limit values …
Export parameters	Manual export: **File	Export** Automatic export: **File	Export Setup**

5.5.3 Well Selection

Before defining the measurement sequence, you have to select the wells of the microplate you want to measure and those which are to receive an injection.

- ❒ To edit an existing parameter file, open this file (**File | Open**).
- ❒ To create a new parameter file, select **Edit | Reset** in the main window. **Untitled.par** is displayed as temporary file name in the bottom right corner of the screen.
- ❒ Select **Options | Read** to open the **Options Read** dialog box.

Neues Bild mit englischer Software!

Figure 5-26: Options Read dialog box

Driver Selection

Device From the **Device** drop-down list, select **BertholdTech Mithras** to define the measurement sequence for ***Mithras***.

Version Shows the driver version. This entry cannot be edited.

Description Describes the selected driver.

- ❒ As soon as you have set up the ***Mithras*** driver, click the <**Options**> button to open the **Options** dialog box with the **Samples** and **Measurement** tabs (Figure 5-27).

On the **Samples** tab, select the plate type and the wells to be measured and to receive an injection.
The measurement sequence is defined on the **Measurement** tab.

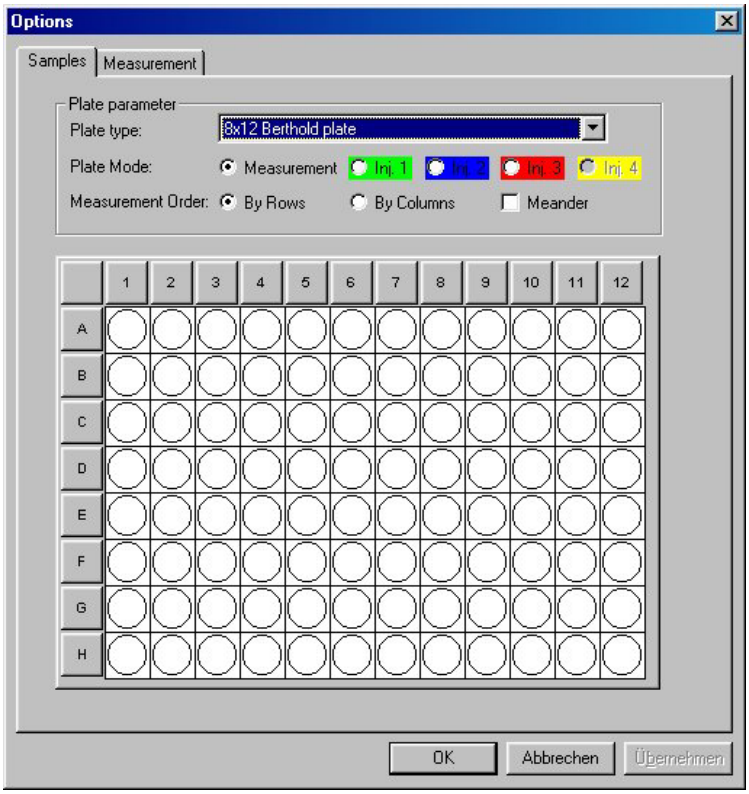


Figure 5-27: Options dialog box, Samples tab

Samples tab

Plate parameter

Plate type

Select the desired plate type with 12*, 24*, 48*, 96*, 384* or 1536* wells. The plate matrix is displayed showing the respective number of wells (*only with option automatic plate height adjustment).

Plate Mode

Select the wells to be measured with the cursor. The active **Plate Mode** indicates for which operation the wells are to be selected. First, activate the **Measurement** mode and with the cursor select the wells to be measured. Then enable the injector (**Inj. 1**, **Inj. 2, Inj. 3 or Inj. 4**) and select the wells to receive an injection by the enabled injector. Up to four injections can be defined for each well. Injections can be selected only for those wells which have already been selected for measurement.
Only injectors installed in the software can be selected (see **Installation | Driver**, chapter 5.2.3).

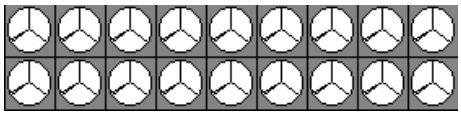
Measurement Order

By Row / By columns Choose if the measurement sequence is to be processed by rows or by columns and within this selection meandering.

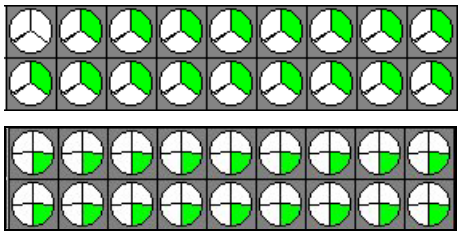
Plate matrix A schematic presentation of a microplate is displayed showing the selected microplate type. Select the wells to be measured and to receive an injection. Depending on the defined operation, the selected wells are depicted differently, so that you can see at one glance for which operation(s) the wells have been selected.

Presentation of selected wells

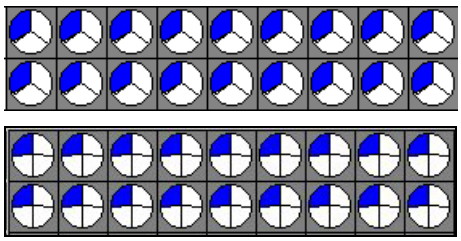
Wells which have been selected for **measurement** are divided into 4 segments against a gray background:



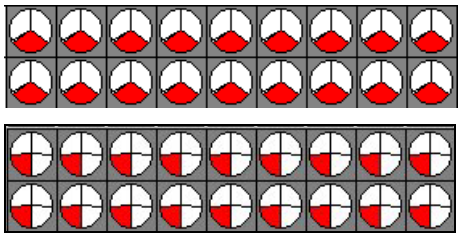
For wells selected for **injector 1** the right segment or the segment in the bottom right corner is green:



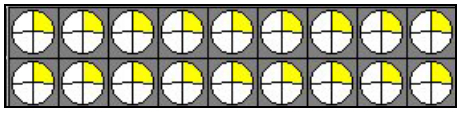
For wells selected for **injector 2** the left segment or the segment in the top left corner is blue:



For wells selected for **injector 3** the bottom segment or the segment in the bottom left corner is red:



For wells selected for **injector 4** the segment in the top right corner is yellow:



Well selection

For well selection, you first have to select the mode (first select **Measurement**); then select the wells with the cursor.

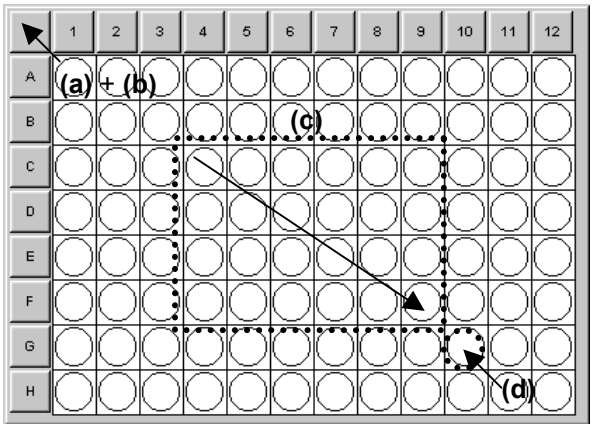


Figure 5-28: Well selection

(a) All wells on a plate

Click on the empty button in the top left corner (between A and 1). All wells on the microplate are selected in accordance with the pre-defined mode.

(b) Injections into all wells selected for measurement

Select the injector and click on the empty button in the top left corner (between A and 1). All wells selected for measurement are in addition selected for the pre-selected injector.

(c) Selected well area

With the mouse button held down, drag the mouse pointer diagonally across the desired well area and then release the mouse button. The wells inside this rectangle are highlighted. You can define several independent rectangular well areas on one plate.

(d) Single wells

Click on the desired well.

(e) *Clear selection*

Clear all: Click right mouse button and select **Clear** from the context menu.
Undo function selected last: Select last function once more, i.e. enable the respective **Plate Mode** and click once more on the wells you want to deselect or click on the empty button in the top left corner (between A and 1).

5.5.4 Definition of Measurement Sequence

The measurement sequence is defined in the **Options** dialog box on the **Measurement** tab.

First, select the wells for measurement and injection on the **Samples** tab; then define the measurement sequence with the individual operations. To do this, select the **Measurement** tab.

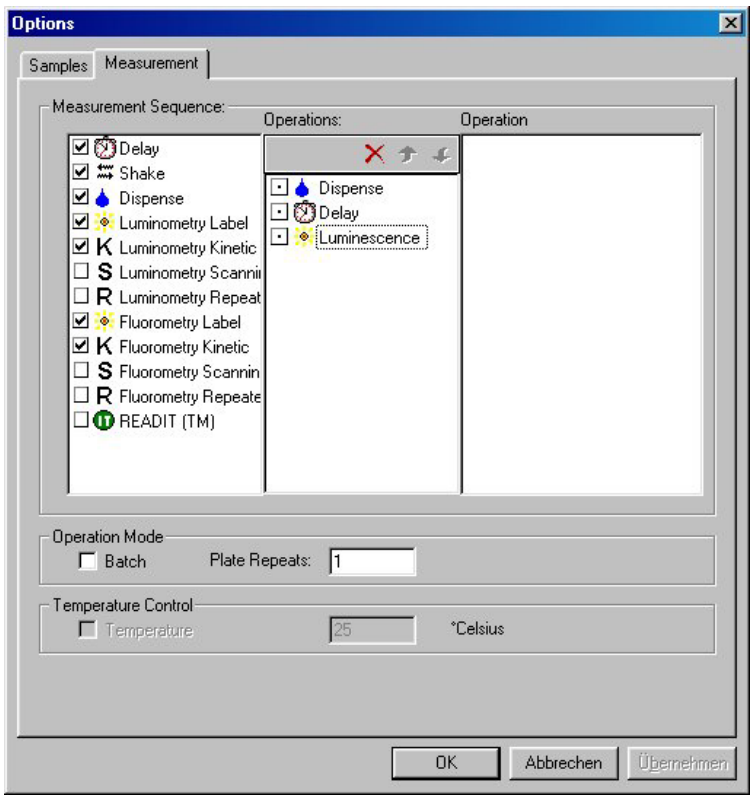


Figure 5-29: Measurement tab

Left column

Shows the available operations. Steps that can be selected are identified by a checkmark. You can select only those steps that are supported by the instrument and the pre-selected measurement mode. For example, if you did not select any wells for injection on the **Samples** tab, the **Dispense** step does not have a checkmark. This ensure that the measurement sequence does not include any operations that cannot be carried out.

***Operations** column*

Operations required for the measurement sequence are copied from the left column into the center column and arranged in the desired order. Individual operations can be copied for the measurement sequence and can be moved to any position.

Click on an operation to show the respective parameters in the **Operation** column.
Double-click on an operation to open the associated parameter dialog box to edit the parameters.

Copy
To copy an operation to the center column, double-click on the respective operation in the left column. This opens the parameter window. Enter the required parameters and confirm the entries with <**OK**>. The window is closed and the operation is entered in the center column behind the last one (chapter 5.5.5).

Select order
In the **Operations** column the selected operations are first listed in the order of their selection. To change the order, click on the buttons  and  . Select the operation which you want to move to another position and click on the respective button until you have reached the desired position. The arrows on both buttons indicate whether the operation is moved up or down.

Clear operation
To delete an operation from the measurement sequence, select this operation and then click on the  button.

***Operation** column*

This column shows the parameters of an individual operation selected in the center column (see Figure 5-26).

Operation Mode

Select the desired mode:

Batch:

In this mode several plates can be measured one after the other using *the same protocol*.
Measurement sequence: After it has been processed, the first plate is moved out of the instrument. Load the next plate and start measurement with the same parameters.

Repeat:

The same plate can be measured several times in succession. Define the number of repeats here.

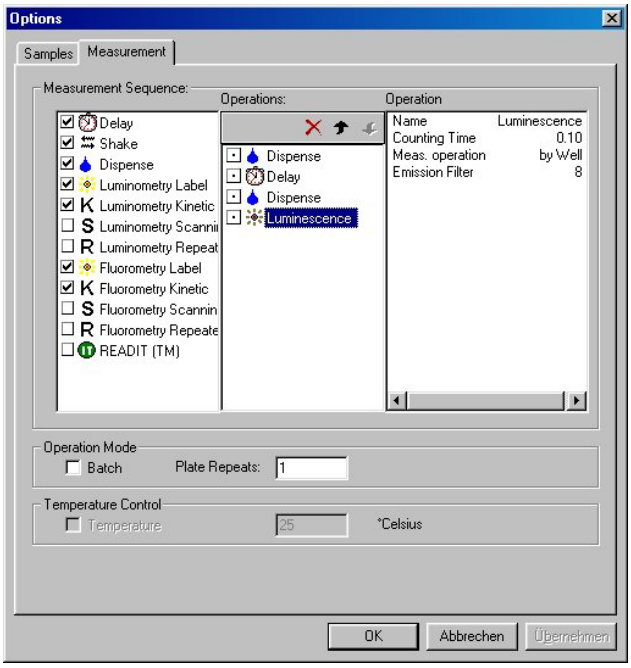


Figure 5-30: Defined measurement sequence with display of reading parameters

Temperature Control

Define the desired temperature if your instrument includes a temperature control (input option: 15° - 45°C).
Please note: You can enter a target temperature in the instrument which is *at least* +5°C above room temperature.

Proceed as follows to define a new measurement sequence:

- ❒ Select **Edit | Reset**. **Untitled.par** is displayed as temporary file name in the bottom right corner of the status bar.
- ❒ Select **Options | Read** to open the **Options Read** dialog box.
- ❒ Select **BertholdTech Mithras** from the device drive list.
- ❒ Click <**Options**> to open the **Options** dialog box with the **Samples** and **Measurement** tab.
- ❒ Select the plate type on the **Samples** tab.
- ❒ In the row **Plate Mode**, enable the item **Measurement** and then select the wells for measurement.
- ❒ Select injector in the **Plate Mode** row and then select the wells into which the selected injector is to dispense.
- ❒ After selection of the wells, select the **Measurement** tab and define the measurement sequence with the individual operations.
- ❒ Copy these operations one after the other to the **Operations** column: in the left column double-click on the desired operation and enter the parameters in the respective window.
- ❒ Confirm your entries with <**OK**>. The respective operation is entered in the **Operations** column behind the operation defined last.
- ❒ In the **Operations** column, the selected operations are first displayed in the order of their selection. Click the buttons  and  to change the order, click  to delete an operation.
- ❒ The parameters of the operation selected in the **Operations** column are displayed in the **Operation** column.
- ❒ Select the operation mode.
- ❒ Enter the temperature, if the instrument includes temperature control and temperature control is needed for the measurement sequence.
- ❒ Confirm entries with <**OK**>.

5.5.5 Operations and their Parameters

Double-click on an operation in the left column to open the respective properties dialog box. Click <**OK**> to accept the entries and append the respective operation to the last operation defined in the **Operations** column.

Double-click on an operation in the **Operations** column to open the properties dialog box and edit the entries. Click <**OK**> to accept the entries.

By plate / By wells

The measurement mode **By plate** or **By wells** has to be selected for nearly all operations. **By plate** means that a defined operation is first performed for all selected wells of the plate, before the next step starts. **By wells** means that all consecutive operations are first performed for the 1st well, then for the 2nd well, etc.

Perform on first plate repeat only

If you choose **Perform on first plate repeat only**, the respective operation is performed only once at the start of a series of repeat measurements (see also **Repeat**).

Delay Delay before an operation

Double-click on Delay to open the **Delay properties** dialog box and define the delay between two operations.

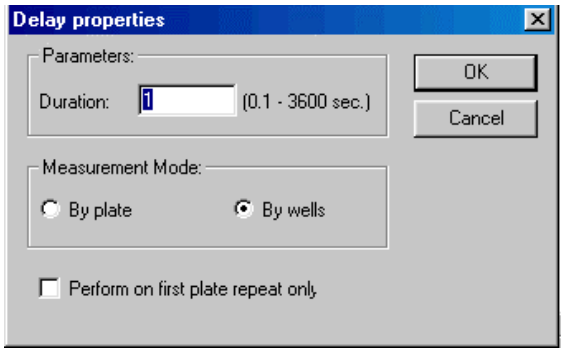


Figure 5-31: Delay properties dialog box

Duration Define delay between 1 and 3600 seconds.

Measurement Mode This function can be used in the plate or well mode.

Shake

Double-click on Shake to open the **Shake properties** dialog box and define the parameters for microplate shaking.

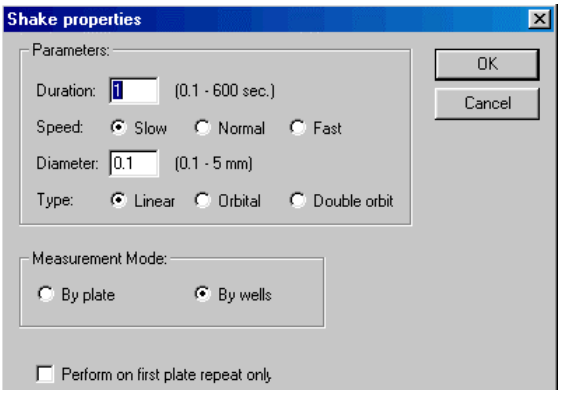


Figure 5-32: Shake properties dialog box

Duration	Define shake duration in seconds.
Speed	Define shake speed. You may choose **Slow**, **Normal** or **Fast**.
Diameter	Define amplitude of shake motion.
Type	Select shake mode: linear, orbital or double orbital.
Measurement Mode	This function can be used in the plate or well mode.

Dispense

Double-click on Dispense to open the **Dispense properties** dialog box. This operation is available only if wells have been selected for injections on the **Sample** tab.

Please keep in mind that you have to define this operation separately for each injector!

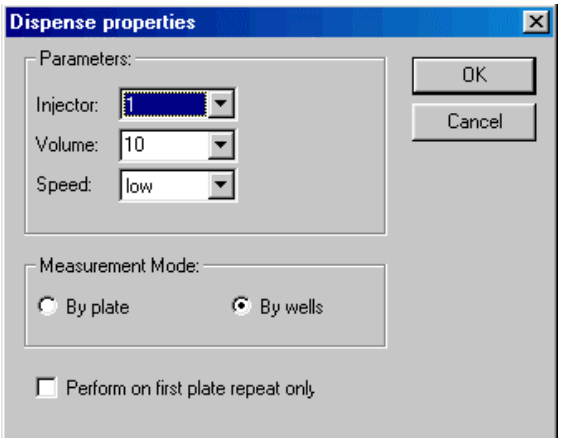


Figure 5-33: Dispense properties dialog box

Injector — Select injector (no. 1, 2, 3 or 4). Only those injectors are displayed for which wells have been selected on the **Samples** tab.

Volume — Define volume to be dispensed (10 to 100 µl).

Speed — Define injection speed: **low**, **middle**, **high**.

Measurement Mode — This function can be used in the plate or well mode.

Luminometry Label Luminescence measurement

Double-click on Luminometry Label to open the **Luminometry**, dialog box and enter the parameters for measurement .

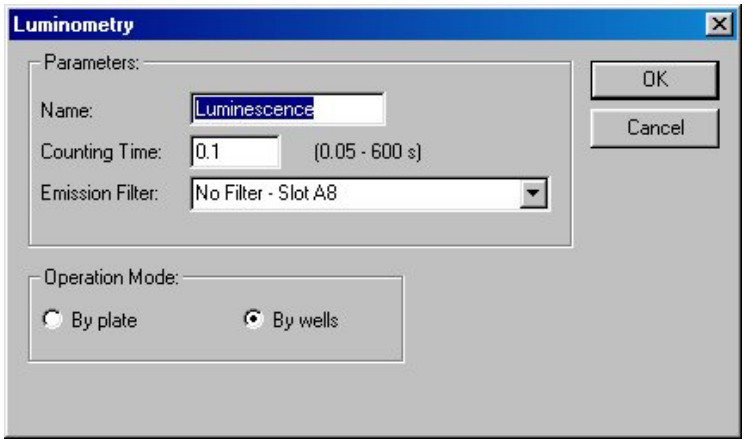


Figure 5-34: Definition of reading parameters

Name Name of measurement.

Counting Time Duration of measurement in seconds.

Emission Filter Select the filter to be used. Usually, no filter is used for luminescence measurements; therefore, filter slot 8 (No Filter) is defaulted.

Measurement Mode This function can be used in the plate or well mode.

K Luminometry Kinetic Kinetics measurement (Luminescence)

Double-click on K Luminometry Kinetic to open the **Kinetics properties** dialog box.
A kinetics measurement always takes place in the **by wells** mode.

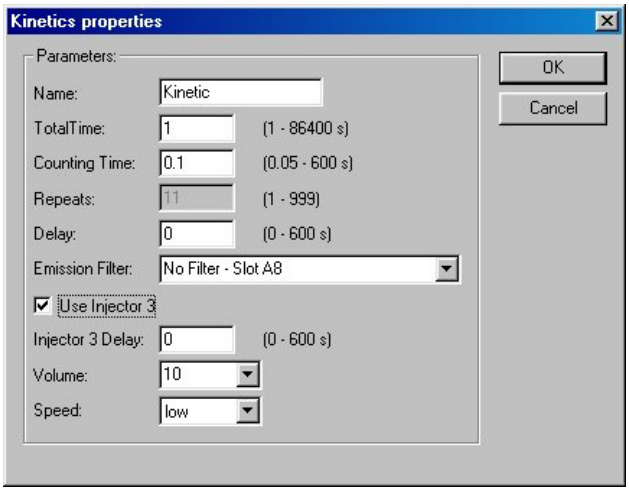


Figure 5-35: Kinetics properties dialog box

Name Name of kinetics measurement.

TotalTime Define total counting time for each selected well.

Interval Define interval counting time of a kinetics sequence of one well.

Repeats The number of data points per well is automatically calculated from the parameters defined (including **delay**). Click <**OK**> to start calculation. The calculated value is displayed on the tab in the **Operation** column.

Delay Delay between single measurements of a kinetics sequence of one well (this function is not yet available).

Emission Filter Select the filter to be used. Usually, no filter is used for luminescence measurements; therefore, filter slot 8 (No Filter) is defaulted.

Use injector 3 Enable injector 3 to perform an injection in reading position during on-going kinetics.

Injector delay Time (counted from the start of the kinetics measurement), after which the injection should take place within the on-going kinetics.

Volume Define volume to be dispensed (inj. 3).

Speed Define injection speed: **low**, **middle**, **high**.

Scanning Luminescence measurement

Double-click on Scanning to open the **Scan properties** dialog box.
Scanning always takes place in the **by wells** mode.

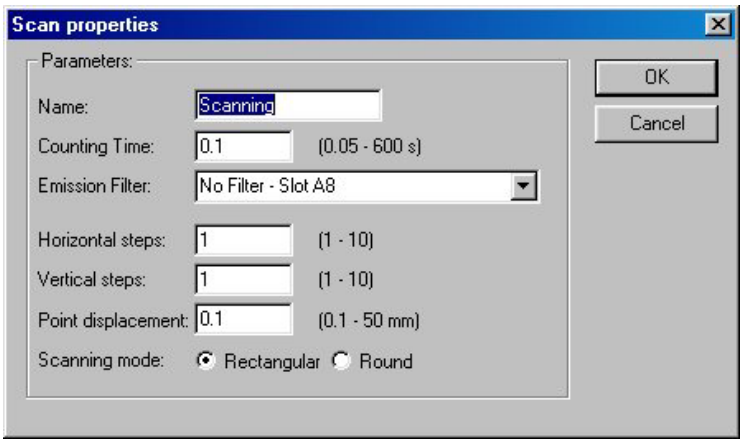


Figure 5-36: Scan properties dialog box

Name Name of scan.

Counting Time Define individual counting time.

Emission Filter Select the filter to be used. Usually, no filter is used for luminescence measurements; therefore, filter slot 8 (No Filter) is defaulted.

Horizontal steps Number of horizontal steps (direction 1...12).

Vertical steps Number of vertical steps (direction A...H).

Point displacement Step width = distance between data points; selectable distances: 0.1 to 50 mm.

Scanning mode: Rectangular or round can be selected. We recommend to select **round** for microplates with round wells, since then the data points in the corner are automatically skipped.

During scanning, one well with up to 100 data points can be measured.
To measure the well of a 96-well microplate (∅ 6 mm) with 100 data points evenly and completely you have to define the following parameters: horizontal and vertical steps: 10 each; point displacement : 0.6 mm.
However, you may also focus on the center of one well and select smaller distances.

R Luminometry Repeat Repeated measurement (Luminescence)

Double-click on R Luminometry Repeat to open the **Repeated** dialog box.

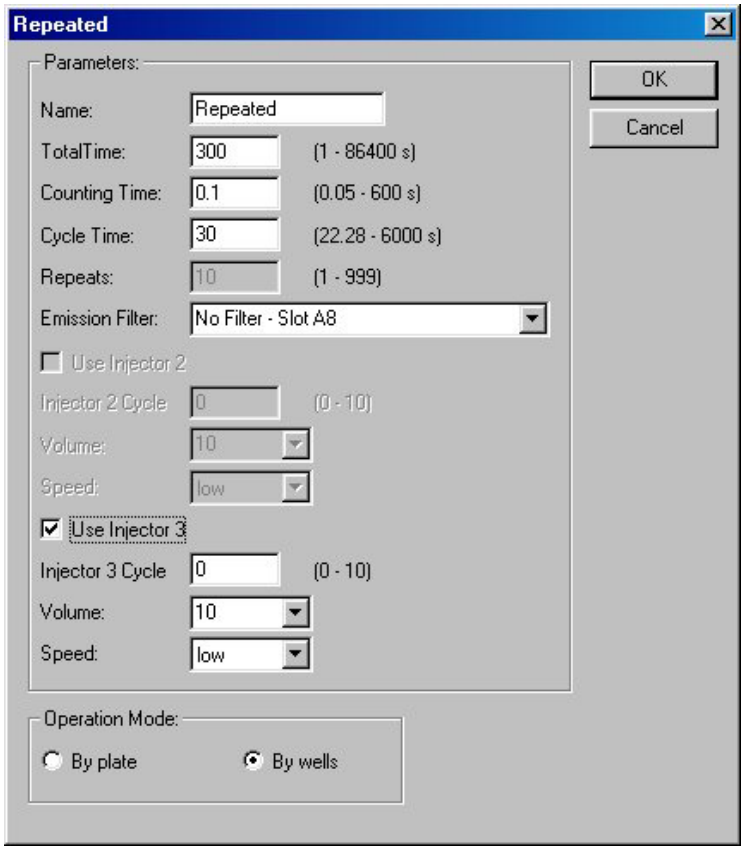


Figure 5-37: Repeated dialog box

Name	Name of repeated measurement.
TotalTime	Define calculated total length of measurement.
Counting Time	Define interval counting time of a repeated measurement per well.
Cycle Time	Define the time of a reading cycle.
Repeats	Define the number of repeats per well (automatically calculated from the previous data and the selected number of wells)
Emission Filter	Select the filter to be used. Usually, no filter is used for luminescence measurements; therefore, filter slot 8 (No Filter) is defaulted.
Use Inj. 2 / Use Inj. 3	Enable injector 2 or 3 to perform an injection in reading position during repeated measurement.
Injector ... Cycle	Define the repeated cycle at which the injection is to take place within the ongoing kinetics.
Volume	Define volume to be dispensed (inj. 2 or 3).
Speed	Define injection speed: **low**, **middle**, **high**.
Measurement Mode	This function can be used in the plate or well mode.

Repeated operation allows optimized execution of kinetics measurements over longer periods of time.

Both operations **Dispense** and **Repeated** have to be used in the **By wells** mode if during a repeated measurement an injection triggering the reaction and immediately thereafter the first measurement should take place.

However, if all selected wells are first to be primed with reagent and the measurement series is to start thereafter, you have to set both operations **Dispense** and **Repeated** to the **By plate** mode.

Fluorometry Label Fluorescence measurement

Double-click on Fluorometry Label to open the **Fluorometry** dialog box for entry of the parameters for the fluorescence measurement .

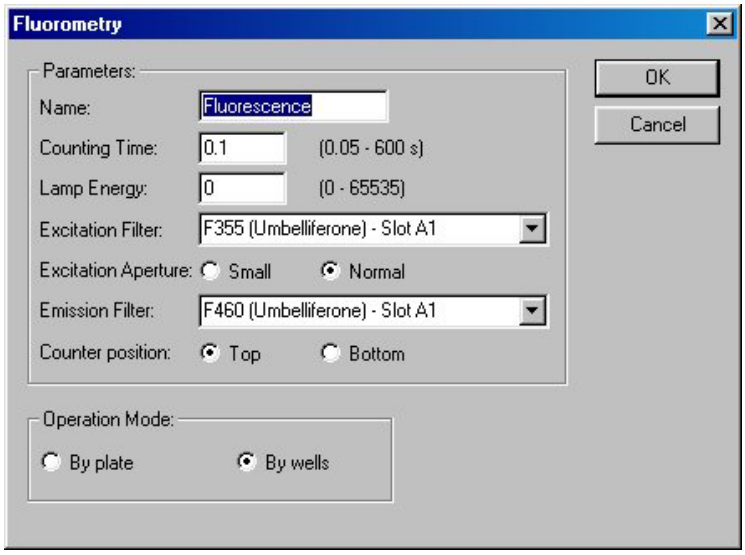


Figure 5-38: Fluorometry dialog box

Name Name of measurement.

Counting Time Counting time in seconds.

Lamp Energy Enter a value between 0 and 65 535. This range corresponds to 0 to 75 Watts and is divided in digital steps.

Excitation Filter Select the desired excitation filter. The pre-defined filter with name and position in the filter slide is displayed.

Excitation Aperture Select the desired width of the excitation beam: small = 1.2 mm; normal = 2.0 mm.

Emission Filter Select the emission filter to be used. The pre-defined filter with name and position in the filter wheel is displayed.

Counter position Select if a fluorescence measurement should take place at the top or bottom. ***Please keep in mind***: injections in reading position for measurement at top is possible only with injector 4.

Operation Mode This function can be used in the plate or in the well mode.

Fluorometry Kinetic Kinetics measurement (Fluorescence)

Double-click on Fluorometry Kinetic to open the **Kinetics properties** dialog box.
A kinetics measurement always takes place in the **By wells** mode.

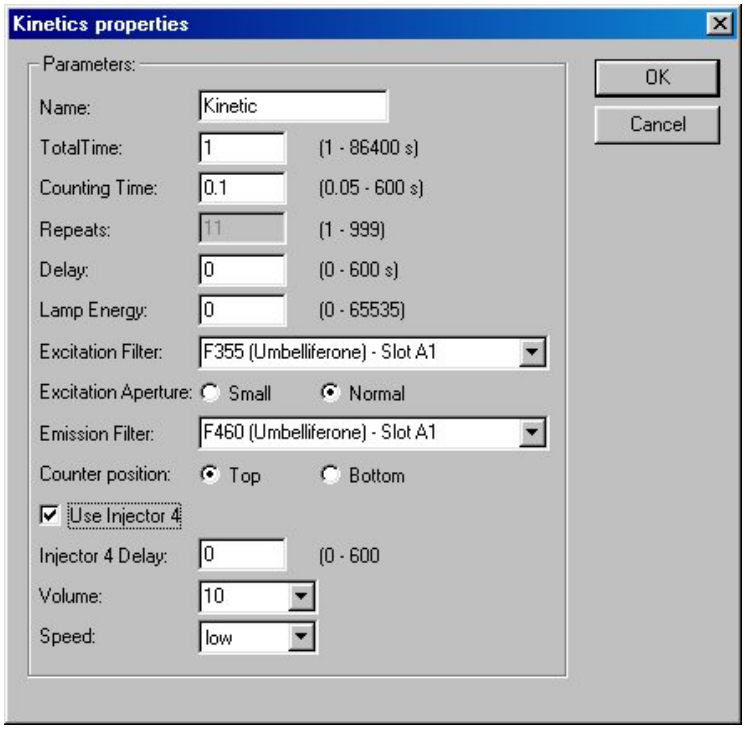


Figure 5-39: Kinetics parameters

Name Name of kinetics measurement.

TotalTime Specify the total counting time for each selected well.

Counting Time Define the individual counting time of a kinetics sequence of one well.

Repeats The number of data points per well is automatically calculated from the entered parameters (including the **Delay**). Calculation takes place as soon as you click <**OK**>. The calculated value is then displayed on the table in the **Operation** column.

Delay Delay time between individual measurements of a kinetics sequence of one well (presently not implemented).

Lamp Energy Enter a value between 0 and 65 535. This range corresponds to 0 to 75 Watts and is divided in digital steps.

Excitation Filter Select the excitation filter. The pre-defined filter with name and position in the filter slide is displayed.

Excitation Aperture	Select the width of the excitation beam: small = 1.2 mm; normal = 2.0 mm.
Emission Filter	Select the emission filter to be used. The pre-defined filter with name and position in the filter wheel is displayed.
Counter position	Select if a fluorescence measurement should take place at the top or bottom. ***Please keep in mind***: injections in reading position for measurement at top is possible only with injector 4.
Use Injector 4	Enable injector 4 to perform an injection in counting position during the ongoing kinetics.
Injector Delay	Time (counted from start of kinetics measurement) after which the injection should take place within the ongoing kinetics.
Volume	Define the volume to be injected (Inj. 4).
Speed	Define the injection speed: **low**, **middle**, **high**.

Fluorometry Scannin Scanning (Fluorescence)

Double-click on Fluorometry Scannin to open the **Scan properties** dialog box.
Scanning always takes place in the **By wells** mode.

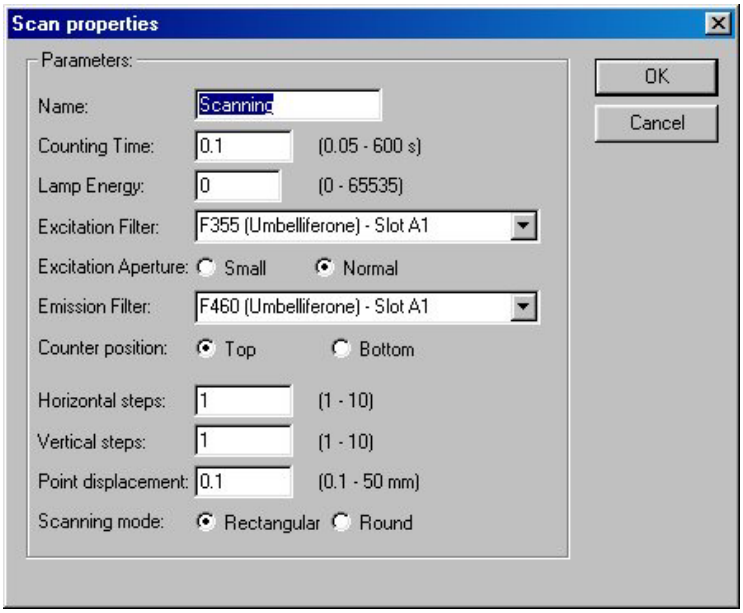


Figure 5-40: Scan properties dialog box

Name — Name of scan.

Counting Time — Define individual counting time.

Lamp Energy — Enter a value between 0 and 65 535. This range corresponds to 0 to 75 Watts and is divided in digital steps.

Excitation Filter — Select the excitation filter. The pre-defined filter with name and position in the filter slide is displayed.

Excitation Aperture — Select the width of the excitation beam: small = 1.2 mm; normal = 2.0 mm.

Emission Filter — Select the emission filter to be used. The pre-defined filter with name and position in the filter wheel is displayed.

Counter position — Select if a fluorescence measurement should take place at the top or bottom. ***Please keep in mind***: injections in reading position for measurement at top is possible only with injector 4.

Horizontal steps — Number of horizontal steps (direction 1...12).

Vertical steps — Number of vertical steps (direction A...H).

Point displacement — Step width = distance between data points. Selectable distances: 0.1 to 50 mm.

Scanning mode:

Rectangular or round can be selected. We recommend to select **round** for microplates with round wells, since then the data points in the corner are automatically skipped.

During scanning, one well with up to 100 data points can be measured.
To measure the well of a 96-well microplate (∅ 6 mm) with 100 data points evenly and completely you have to define the following parameters: horizontal and vertical steps: each 10; point displacement : 0.6 mm.
However, you may also focus on the center of one well and select smaller distances.

Fluorometry Repeat Repeated measurement (Fluorescence)

Double-click on Fluorometry Repeat to open the **Repeated** dialog box.

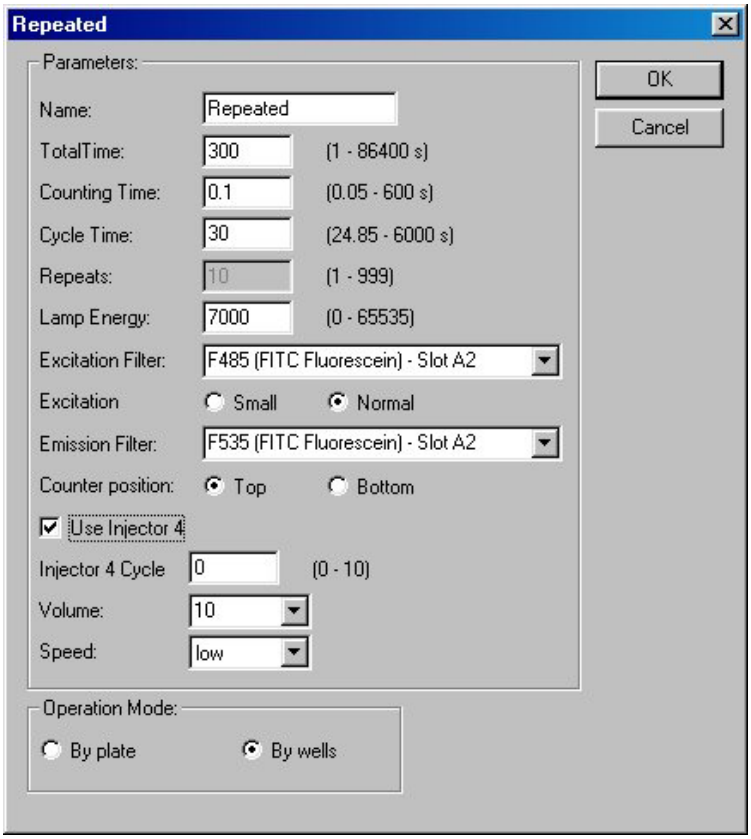


Figure 5-41: Repeated dialog box

Name Name of repeated measurement.

TotalTime Define the calculated total length of the measurement.

Counting Time Define the individual counting time of a repeated measurement per well.

Cycle Time Define the cycle time.

Repeats Define the number of repeats per well.

Lamp Energy Enter a value between 0 and 65 535. This range corresponds to 0 to 75 Watts and is divided in digital steps.

Excitation Filter Select the excitation filter. The pre-defined filter with name and position in the filter slide is displayed.

Excitation Aperture Select the width of the excitation beam: small = 1.2 mm; normal = 2.0 mm.

Emission Filter Select the emission filter to be used. The pre-defined filter with name and position in the filter wheel is displayed.

Counter position Select if a fluorescence measurement should take place at the top or bottom. ***Please keep in mind***: injections in reading position for measurement at top is possible only with injector 4.

Use Injector 4 Enable injector 4 to perform an injection in counting position during the ongoing kinetics.

Injector 4 Cycle Define the cycle in which the injection should take place within the ongoing kinetics.

Volume Define the volume to be injected (Inj. 4).

Speed Define the injection speed: **low**, **middle**, **high**.

Operation Mode This function can be used in the plate or in the well mode.

Repeated operation allows optimized execution of kinetics measurements over longer periods of time.

Both operations **Dispense** and **Repeated** have to be used in the **By wells** mode if during a repeated measurement an injection triggering the reaction and immediately thereafter the first measurement should take place.

However, if all selected wells are first to be primed with reagent and the measurement series is to start thereafter, you have to set both operations **Dispense** and **Repeated** to the **By plate** mode.

READIT (TM) READIT™ measurement

The Readit™ operation allows you to run the Readit™ tests by Promega for SNP determination. This operation comprises sequential measurement of 3 microplates and automatic entry of the measured data into the EXCEL spreadsheet „tier2master.xls“.

Double-click on READIT (TM) to open the **Readit** dialog box.

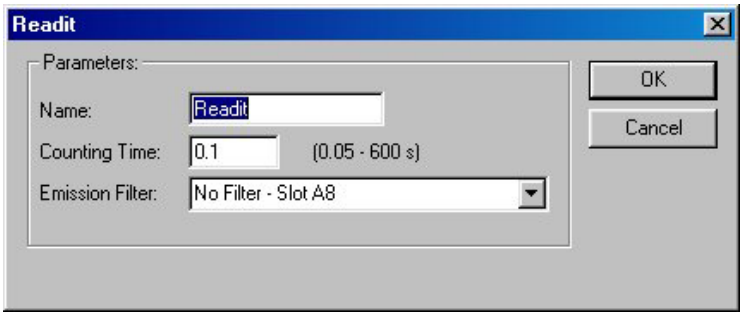


Figure 5-42: Readit dialog box

Name Name of Readit™ measurement.

Counting Duration of measurement in seconds.

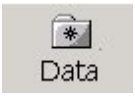
Emission Filter Select the emission filter to be used. The pre-defined filter with name and position in the filter wheel is displayed here.

5.5.6 Definition of Evaluation Parameters in Child Windows

Evaluation parameters are selected using items on the **navigation bar** or via **tool buttons** and defined on the respective matrices. In general, default settings are defined such that they hardly need to be changed for simple luminescence measurements. Check the following matrices anyway and, if necessary, edit the settings.
You may add more matrixes in each menu item (via **Options | Matrix...**) and define parameters for each matrix for measurement and evaluation (by well, by row or column or for the entire microplate).
To edit matrices, double-click on the respective tab. For more information please refer to the ***MikroWin2000*** User Guide.

Child windows

Data child window



The data supplied by the Reader Unit are entered on matrix 1. You may enter sample ID, error information and dilution factor on the defaulted matrices.

Template child window

Here you may change the partition of the sample matrix and define controls and standards. The other matrices are partitioned accordingly.

Calculation child window

Three matrices show the well positions (**Sample_ID**), the measurement data (**Reader_Values**) and the calculated results (**Results**). Any formula may be used for measurement data and results. You can type a formula directly into the calculation formula text box or select it from the **Add Formula** dialog box.

A formula can be defined for individual wells, columns or rows or for the entire plate.

Select formula:

❒ Select the desired tab in the **Calculation** child window and click on Add Formula. The **Add Formula** dialog box opens.

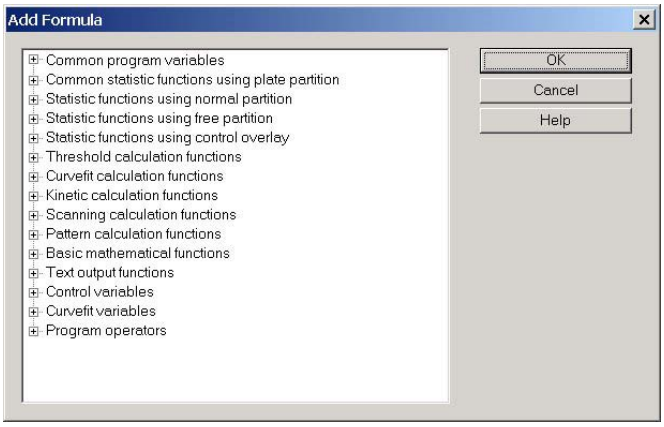


Figure 5-43: Add Formula dialog box

❒ Click on the plus sign (**+**) of the desired formula type to display the list of available formulas.

❒ Select the formula you want from the formula list and click <**OK**>. The formula is entered into the **Calculation formula of Position...** text box. Please consult the respective chapter in the MikroWin2000 User Guide.

Figure 5-44: Calculation formula text box

❒ Define the plate area to which the formula is to be applied:

For the entire plate: Click on button **#** .

For a column or row: Click on the respective **column** or **row button**.

For a single well: First select the well, then select the formula and place the cursor into the formula text box. Then push the <**Return**> key.

Results child window

The displayed matrices are identical with those defined in the **Calculation** child window. Here, the data and calculated values are displayed. These matrices are used depending on their numbering for export of the file (see chapter 5.2)

5.5.7 Definition of Export Parameters

You have to define export parameters if you want to export data to an Ascii or Excel file. Prerequisite is that the export driver(s) has/have been installed and set up accordingly (see chapter 5.2).

Please refer to chapter 5.3.2 to define automatic data export after each measurement.

Please see chapter 5.3.1 to perform manual data export.

5.5.8 Saving Parameter Files

Select **File | Save** to save the active parameter file.
Select **File | Save As** to save the file under a new name or to create a new parameter file („**Untitled.par**“).
See also chapter 5.5.2.

5.5.9 Basic Parameter Files

Fifteen different basic parameter files are supplied for the most common types of measurement. Different print formats are associated with the individual parameter files. The following basic configurations are included:

BRET-Assay – Bret.par

Injectors	No injector
Well selection	All wells for measurement
Measurement sequence	**Measurement 1** (Renilla Luciferase) with emission filter no. 3 (by well), 1 second.
	Measurement 2 (eYFP)) with emission filter no. 4 (by well), 1 second
***Calculation** child window*	**Sample**: POS = position of cavity
	RLuc: raw data of measurement 1 **eYFP**: raw data of measurement 2 **Ratio**: measurement 2/measurement 1

BRET-Assay – Bret2.par

Injectors	No injector
Well selection	All wells for measurement
Measurement sequence	**Measurement 1** (Renilla Luciferase) with emission filter no. 5 (by well), 1 second.
	Measurement 2 (GFP) with emission filter no. 6 (by well), 1 second.
***Calculation** child window*	**Sample**: POS = position of cavity
	DeepBlueC: raw data of measurement 1 **GFP2**: raw data of measurement 2 **Ratio**: measurement 2/measurement 1

Fluorescence measurement – Fluorescein.par

Injectors	None
Well selection	All wells for measurement and injection
Measurement sequence	**Measurement**: 0.1 second, by plate **Lamp energy**: 7000 **Excitation filter** no. 2, **Emission filter** no. 2 **Counter position**: Top
***Calculation** child window*	**Sample**: POS = position of cavity **Result**: average value **Plate**: limit value calculation

Fluorescence kinetics measurement – Fluorescein kin10.par

injectors	Injector no. 4
Well selection	All wells for measurement and injection
Measurement sequence	**Injection**: 100 µl, middle speed, by well **Measurement**: 10 seconds total time 1 second interval time 11 data points No delay **Lamp energy:** 7000 **Excitation filter** no. 2, **Emission filter** no. 2 **Counter position**: Top
***Calculation** child window*	**Sample**: POS = Position of cavity **Peak**: Maximum of kinetics measurement **Integral**: Integral of kinetics measurement **T.Slope**: Time at maximum slope **T.Max**: Time at maximum Time at maximum of kinetics

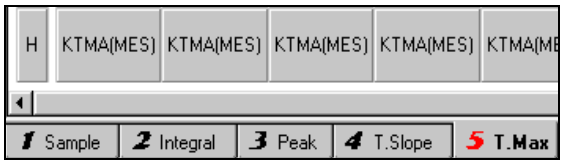


Figure 5-45: Matrices in the Calculation child window

Fluorescence repeated measurement - Fluorescein Rep10.par

Injectors	Injector no. 4
Well selection	All wells for measurement and injection
Measurement sequence	**Injection**: 100 µl, low speed, by well
	Measurement: 0.1 second, by well
	Lamp energy: 7000
	Excitation filter no. 2, **Emission filter** no. 2
	Counter position: Top
	Cycle time: 30 seconds
	Total time: 300 seconds

Luminescence measurement – Lumi1s.PAR

Injectors	Injector 3 (reading position)
Well selection	All wells for measurement and injection
Measurement sequence	**Injection**: 100 µl, middle speed, by well
	Measurement: 1 second, by well
	Emission filter no. 8

***Calculation** child window*

Sample: POS = position of cavity
Reader: raw data
Result: average of matrix 2 (Reader)

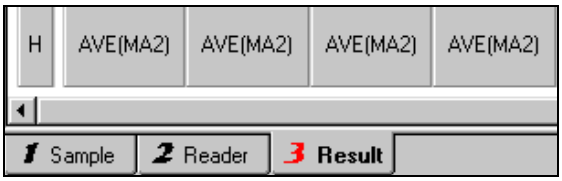


Figure 5-46: Matrices in Calculation child window

Luminescence measurement without injection – Lumi_01s.PAR

Well selection	All wells for measurement and injection
Measurement sequence	**Measurement**: 1 second, by well **Emission filter** no. 8
***Calculation** child window*	**Sample**: POS = position the cavity **Reader**: raw data **Result**: average of matrix 2 (Reader)

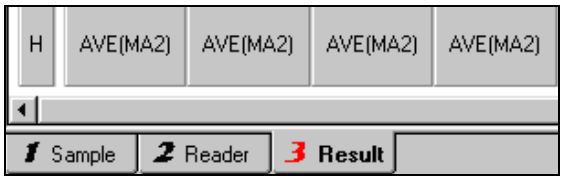


Figure 5-47: Matrices in Calculation child window

Kinetics measurement – Lumi 1s Kin10.PAR

Injectors	Injector 3 (reading position)
Well selection	All wells for measurement and injection
Measurement sequence	**Injector 3**: 100 µl, middle speed, by well **Delay**: 0 second **Measurement**: 10 seconds total time 1 second interval time 11 data points no delay **Measurement mode**: by well **Emission filter** no. 8
***Calculation** child window*	**Sample**: POS = position of cavity **Peak**: Maximum of kinetics measurement **Integral**: Integral of kinetics measurement **T.Slope**: Time at maximum of slope **T.Max**: Time at maximum of kinetics

Luminescence - Repeated measurement – Lumi 1s Rep10.PAR

Injectors	Injector 3
Well selection	All wells for measurement and injection
Measurement sequence	**Injector 3**: 100 µl, low speed, by well **Measurement**: **Total Time**: 300s **Counting time:** 0.1 seconds **Cycle time**: 30s **Emission filter** no. 8
***Calculation** child window*	**Sample**: POS = position of cavity **Peak**: Maximum of repeated measurement **Integral**: Integral of repeated measurement **T.Slope**: Time at maximum of slope **T.Max**: Time at maximum of kinetics

Figure 5-48: Matrices in Calculation child window

Dual Luciferase Reporter Gene Assay – DLR.PAR

Injectors	Injector 2 and injector 3
Well selection	All wells for measurement and two injections
Measurement sequence	**Injection 1**: 100 µl, low speed, by well **Delay**: 2 seconds **Measurement 1 (Firefly)**: 10 seconds, by well **Injection 2**: 100 µl, middle speed, by well **Delay**: 2 seconds **Measurement 2 (Renilla)**: 10 seconds, by well
***Calculation** child window*	**Sample**: POS = position of cavity **Firefly**: data of measurement 1 (after injection 1) **Renilla**: data of measurement 2 (after injection 2) **Ratio**: data of measurement 1 / data of measurement 2

Figure 5-49: Matrices in Calculation child window

Dual Luciferase Reporter Gene Assay in 384-well microplate – DLR384.PAR

Injectors	Injector 2 and injector 3
Well selection	All wells for measurement and 1 injection
Measurement sequence	**Measurement 1 (Firefly)**: 10 seconds, by well **Injection 1** **Delay 1** **Injection 2**: 100 µl, low speed, by well **Delay**: 2 seconds **Measurement 2 (Renilla)**: 10 seconds, by well
***Calculation** child window*	**Sample**: POS = position of cavity **Firefly**: data of measurement 1 **Renilla**: data of measurement 2 (after injection 2) **Ratio**: data of measurement 1 / data of measurement 2

Figure 5-50: Matrices in Calculation child window

Note: The substrate for the firefly reaction is added manually outside the instrument.

Readit measurements – READIT.PAR

Injectors	Injector 3 (reading position)
Well selection	All wells for measurement and injection
Measurement sequence	**Injection**: 100 µl, high speed, by well **Measurement**: 1 second counting time Measurement mode: by well *Note:* The measurement is carried out sequentially using 3 different microplates. After measurement of a plate, this plate is moved out; unload it and load the next microplate. In addition, the measurement data is automatically entered into the EXCEL spreadsheet „tier2master.xls“.
***Calculation** child window*	**Sample**: POS = position of cavity **Value 1**: raw data of measurement 1 **Value 2**: raw data of measurement 2 **Value 3**: raw data of measurement 3

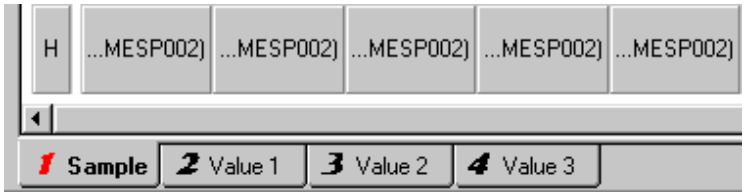


Figure 5-51: Matrices in Calculation child window

5.6 Measurement and Evaluation

1. Prepare instrument

- ❒ If necessary, connect wash solution and wash tubings (**Instrument | Wash**, see chapter 5.4.4.
- ❒ Connect reagent bottle and prime tubings (**Instrument | Prime**, see chapter 5.4.3).

2. Open and edit parameter file

- ❒ Open parameter file (*.par) for measurement (**File | Open**, see chapter 5.5.2.
- ❒ Select **Options | Read** to edit well selection and measurement sequence.
- ❒ You may edit the matrices (**Data**, **Template**, **Calculation**, **Results**).

3. Start measurement

To start the measurement sequence click on the **Read** button in the main window. A status bar is displayed below the matrix with text boxes and buttons for the measurement:

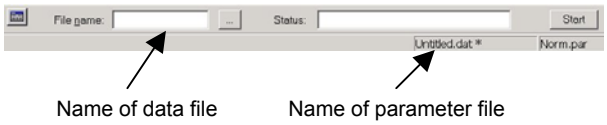


Figure 5-52: Status bar after start of a measurement

The *name* of the parameter file used is displayed in the bottom row (right).

Click  to open the parameter file used and edit the default settings, if necessary. The **Options Read** dialog box opens. Click on <**Options**> to open the **Options** dialog box to view and edit the well selection and the measurement sequence.

File name: Enter a name for the data file. It consists of the name of the parameter file and has the extension ***.dat**. The file name may be overwritten, but the extension cannot be changed.

Click <**Start**> to start the measurement provided you have entered a file name for the data.

As soon as you have clicked on <**Start**> the plate slide moves out of the instrument. On the screen you see the prompt to load a microplate.

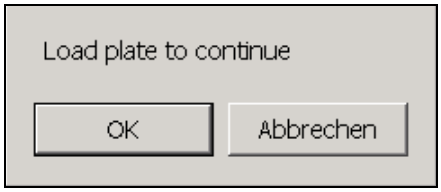


Figure 5-53: Loading a microplate for measurement

Load microplate (with A1 at rear left) and click <**OK**>.
The plate is moved into the instrument and the pre-defined measurement sequence is carried out.
During measurement, you may view the already available data and calculations on the matrices of the **Results** menu (Figure 5-54).
Upon completion of the measurement the plate is ejected.
If you have pre-selected the item **Batch**, the system expects the next microplate which will be measured using the same parameters.

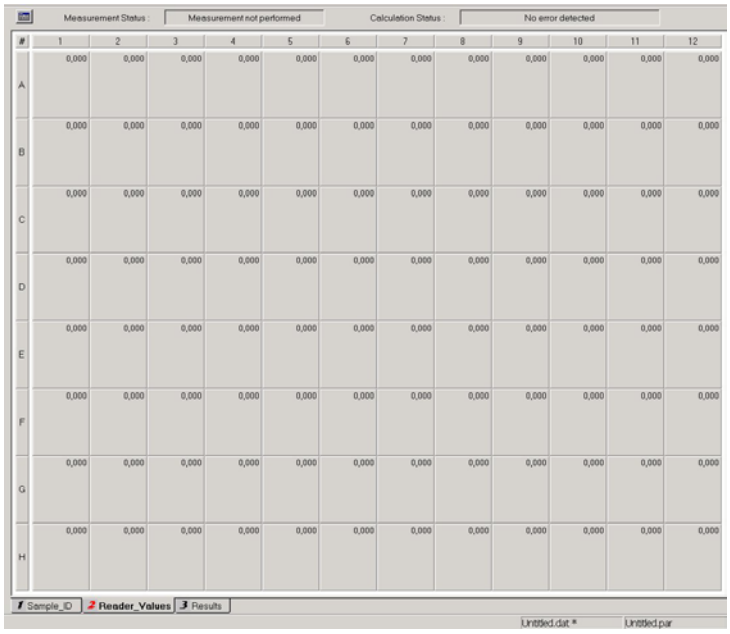


Figure 5-54: On-line presentation of measured data

Results are presented in the **Graphics** menu.

6. Maintenance

6.1 Cleaning the Instrument

Cleaning the instrument surface

The **surface** of the instrument is protected by a washable finish. Dirty or dusty surfaces should be cleaned using a damp cloth. If necessary, use a mild detergent.

Do not use a scouring agent!

Cleaning the inside of the instrument

Before opening the instrument, turn it off and disconnect it from power supply!

Open the screw on the instrument cover to clean the instrument inside. Then raise the cover. If necessary, the photomultiplier can be raised as well.
Always keep the plate slide as well as the inside of the instrument (e.g. below the plate slide) clean. Wipe off any dirt using a damp cloth. Use cotton buds for corners. Remove dirt fairly quickly so it does not get dry and may not have any adverse effect on the movement of the plate slide.

6.2 Cleaning Tubings

Injector tubings have to be washed

- ❒ before starting work
- ❒ before changing reagents
- ❒ at the end of each work session before turning off the instrument
- ❒ after longer periods of inactivity

Use solutions recommended by the kit manufacturer, e.g. distilled water, diluted alcohol, hypochlorite solution ...

Injector tubings have to be primed

- ❒ prior to each measurement using the respective reagents.

Please refer to chapter 5.4.3 and 5.4.4 for more information on washing or priming the tubings.

6.3 Fuse Replacement

The fuse is located on the instrument rear panel in a black fuse holder to the left of the mains switch.

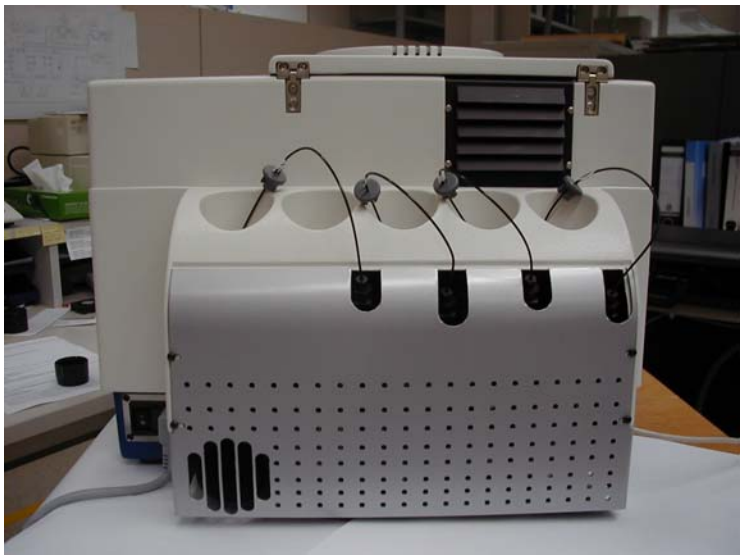


Figure 6-1: Instrument rear panel

Proceed as follows

- ❒ Turn instrument off and pull power cord.
- ❒ Insert screwdriver or similar tool into fuse holder recess and lift it out by applying slight pressure.
- ❒ Take faulty fuses out.
- ❒ Insert new fuses. **Use only the fuse type specified!**
- ❒ Insert fuse holder again.

6.4 Preparations for Transport

The **following safety provisions have to be taken** to transport or ship the instrument:

Preparation for transport of instrument

- ❒ Turn instrument off and disconnect it from mains.
- ❒ Open instrument cover.
- ❒ Move plate tray manually to the position where transport safety screw 2 can be inserted and tightened between the hole in the transport plate. If the screw does not catch, move the height adjustment of the optics down by hand by turning the toothed belt clockwise.
- ❒ Once transport safety screw 2 has been fixed, move the height adjustment of the optics carefully up by hand by turning the toothed belt counter-clockwise.
- ❒ Tighten transport safety screw 2 – ensure through slight sideways motion by a few millimeters that the detector is in the correct position.

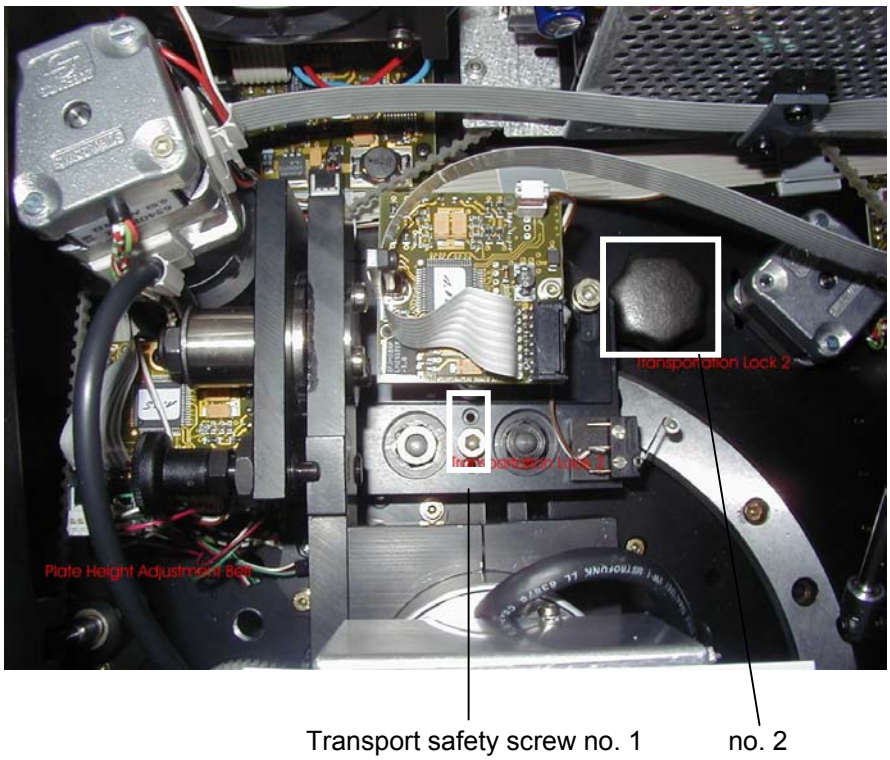


Figure 6-2: Instrument rear panel

7. Technical Data

Operating voltage	100 – 230 V
Frequency	50 / 60 Hz; 180 VA
Power consumption	70 VA
Safety standards	EN 61010-1, EN 61326-1, EN 61000-3-2, EN 61000-3-3 ; EN 61326
Temperature range	Storage: 0° - 40°C Operation: 15° - 35°C
Humidity	10 – 85% no condensation
Dimensions	472 x 494 x 374 mm (W x D x H), including injectors
Weight	52 kg
Detection unit	Low-noise photomultiplier with single photon counting technology (9107 SA)
Reading technologies	Luminescence, TOP reading fluorescence, Bottom reading fluorescence, BRET, FRET
Excitation source	Halogen lamp, 75 W, 340 to 700 nm
Excitation filter	355 nm, 485 nm
Emission filter	460 nm, 535 nm 400 nm, 515 nm, 480 nm, 530 nm (with BRET option)
Sensitivity	< 20 amol ATP < 10 fmol FITC
Dynamic range	> 6 decades
Crosstalk	Low crosstalk due to crosstalk reduction methods: 5 x < 10^{-6}
Injector	Up to 4 injectors (variable volumes: each 10 – 100 µl), *JET* injection technology, injections in plates with up to 384 wells
Plate formats	All microplates with 6 to 1536 wells (external dimensions: 86.0 x 128.2 x 17.0 mm (W x L x H); Petri dish, Terasaki plates, filter
Plate height adjustment	Optional: automatic detection and adjustment to different plate heights (14.0 mm to 25.0 mm)
Temperature control	Optional: +5°C above room temperature to 42°C
Operation modes	Integral measurement 0.1 – 600 second Kinetics measurement (total length up to 24 h) Repeated measurement (total length up to 24 h) Plate repeats (up to 999) Scanning (up to 100 single data points) Dispensing with 4 independent variable injectors Shaking (3 modes, variable amplitude and speed) BRET and BRET² assays Dual luciferase reporter gene assay Readit™ protocol Delay (up to 600 second)

Data evaluation	
Sample parameters	Free plate loading (samples, standards, controls) Allocation of sample identifiers GLP documentation, e.g. lot number Test kit validation formulas
Evaluation	Qualitative cut-off determination with up to 3 categories Kinetics Linking of different types of evaluation Free input of calculation formulas Standard curve calculation (only complete version!)
Result display	Presentation in multi-matrix format or list format Graphical presentation of measured values Integrated statistics functions
Export	Output of all raw data and calculations (manual or automatic) in matrix or list format Output as a file (network connection possible) or to printer
Robotic Integration	Optional
Ports	RS232, 9 pole
Operating system	Win98, Win2000, WinNT, Win XP
PC requirements	Pentium processor, 500 MHz (or better), CD ROM drive, display 1024x768 (or better), serial port, parallel port
Software	Windows® BERTHOLD TECHNOLOGIES MikroWin2000

Order Information

	Order Number
Mithras LB 940 incl. Mikrowin 2000 *Lite*	940-38099-10
Injector 1 pre-position L, 10 – 100 µl	940-37772-11
Injector 2 reading position L, 10 – 100 µl	940-37772-12
Injector 3 reading position L, 10 – 100 µl	940-37772-13
Injector 4 reading position F, 10 – 100 µl	940-37772-14
Waste pump (for injectors 1 to 3)	940-
Plate height adjustment	940-
Robot integration module	940-
Temperature control	940-
Bottom-reading fluorescence	940-
BRET package	940-
MikroWin 2000 full version	37854-025
MikroWin 2000 full version, German language	37854-020

8. Appendix

8.1 Index